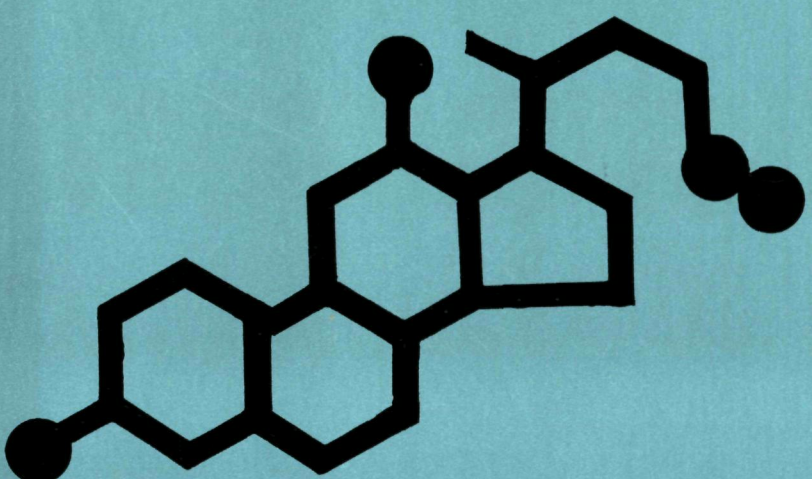
Deoxycholate Metabolism in Man

With Special Reference To Colonic Carcinogenesis



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The studies presented in this thesis were performed in the Division of Gastroenterology, Department of Medicine (head Prof.Dr. C.L.H. Majoor†, at present Prof.Dr. A. van 't Laar), St. Radboud Hospital, University of Nijmegen, Nijmegen, The Netherlands.

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Aan Martha,
Michiel, Erik en Paul

Aan mijn ouders

CONTENTS Page :

CHAPTER I

INTRODUCTION

The discovery that colonic carcinogenesis in man is associated with a western life-style and in particular with certain dietary habits has emerged from epidemiological data of the last four decennia. They demonstrate that not only hereditary factors but also environmental factors are involved.

The search for complete carcinogens such as radiation, air and water pollutants, food additives or viral infections has not yielded many clues. However, studies suggesting that nutrition affects colonic carcinogenesis by endogenous tumour-promoting compounds such as bile-acids appear to be useful leads. The impact of possible complete endogenous carcinogens is less certain.

The main purpose of the work described here is to extend our knowledge of colonic carcinogenesis in man by investigating the metabolism of deoxycholate. This major bacterial bile-salt metabolite in human bile is formed in the large intestine from cholic acid. We are of the opinion that the absorption of deoxycholate from the colon may be an accurate marker of colonic exposure to tumour-promoting bile-acid metabolites in general.

A review will be given in chapter II on the data from the literature on the epidemiology and aetiology of colon carcinoma. Chapter III provides background information and a description of the methodology for studying bile-salt metabolism and intestinal transit time.

Age-dependent differences in healthy subjects (young adults and elderly persons) with regard to deoxycholate and cholate metabolism are the subject of chapter IV.

A comparison of patients with colonic adenomas, where the risk of colon cancer is high, and control subjects with respect to deoxycholate absorption is made in chapter V.

The relation of colonic microenvironment, intestinal transit time and deoxycholate absorption in the large intestine is demonstrated in chapter VI.

In chapter VII we have analysed our data on biliary lipid composition

in healthy subjects to test the hypothesis that colonic absorption of deoxycholate may predispose to the formation of bile supersaturated with cholesterol and to gallstones.

In chapter VIII the findings will be summarized and their implications for colonic carcinogenesis in particular will be discussed.

CHAPTER II

EPIDEMIOLOGY AND AETIOLOGY OF LARGE BOWEL CARCINOMA

2.1. Epidemiology

2.1.1. Incidence and Mortality

The annual mortality caused by colorectal carcinoma is usually expressed per 100.000 inhabitants of a certain age group in order to account for ageing (a possible confounding variable) of a given population. This is a prerequisite for comparisons of average figures from several countries. In a similar way age-adjusted figures can be calculated from the annual number of newly registered cases, i.e. the incidence of colorectal carcinoma.

In the Netherlands, incidence figures of colon cancer are estimated from mortality figures or from registration of hospital diagnoses.

Colorectal carcinoma mortality rates in the Netherlands have shown an increase during the last three decades (see Table)

COLO-RECTAL CARCINOMA IN THE NETHERLANDS ANNUAL MORTALITY BY SEX (PER 100.000)[1]

PERIOD	COLONCARCINOMA		RECTAL CARCINOMA	
	males	females	males	females
1951 - 55	9.7	12.8	8.4	6.2
1956 - 60	10.8	14.0	8.3	6.0
1961 - 65	12.2	15.5	8.3	6.2
1966 - 70	13.5	17.8	8.6	6.7
1971 - 74	14.2	18.2	8.9	6.5
1975 - 78	16.0	19.2	8.9	6.6

It is at present a common malignancy, only second to lung carcinoma in males and breast cancer in females.

Considerable differences in estimated annual colorectal cancer incidence rates in various countries have been noted[2,3]. In Western Europe, including the Netherlands, in the United States, Canada, Australia and New Zealand annual figures of 20 to more than 30 per 100.000 inhabitants are noted.

An incidence of less than 10 is reported from the majority of South American, African and Asian countries including Japan, but with the exception of the Caucasian population. Intermediate figures come from South and East Europe. Remarkably, great variations are seen in Scandinavia: in Denmark over 30, but in the rural areas of Finland below 15.

2.1.2. Differences in incidence between colon carcinoma and rectal carcinoma.

In general, a high incidence of colon carcinoma is associated with a high incidence of rectal carcinoma. However, in countries with a low incidence of colon carcinoma a rather high proportion of all carcinomas of the large bowel are rectal carcinomas[4]. The overall predominance of males in rectal cancer patients and the predilection of disease for persons from low social classes are other distinct features of both cancer sites. In high incidence areas, colon cancer is slightly more frequent in females than in males, only in the over sixty age group is an opposite ratio (but still near to unity) reported[3,4].

2.1.3. Time trends in sex ratio and cancer site in the colon.

Correa and Haenszel recently described an epidemiological model of colorectal carcinoma based on analysis of sex ratio and predilection sites[4]. This model distinguishes an endemic phase of the disease (a pattern occuring in high incidence countries) from an epidemic phase, (the mode of presentation in low incidence countries). Transition from epidemic to endemic phase can be illustrated by time trends in the mode of presentation of colorectal cancer reported from Japan: an early characteristic of increasing incidence is a change of predilection site of colon carcinoma from the right side of the colon to the left side (especially the sigmoid colon). The proportion of rectal carcinomas in the group of large bowel carcinomas as a whole subsequently decreases and finally the predominance of the female sex for colon carcinoma and the male sex for rectal carcinoma subsides.

Since the sex ratio of large bowel carcinoma in general is near to unity much agreement exists on the notion that hormonal influences will play a minor direct role. Recently, much attention has been paid to the increased prevalence of colon carcinoma of the right side of the colon in cholecystectomized women only[5]. Other specific indications that sex or sex hormones play an important modulating role in colonic carcinogenesis are lacking at present.

2.1.4. Migrant population studies

Several comparative studies on the incidence of colorectal cancer among immigrants and that of their country of origin have been carried out in the United States. It is apparent from these studies that immigrants from low incidence areas like East Europe, Asia or Japan show a rise in mortality and morbidity of large bowel carcinoma to the level of the American population when they adapt to the American life-style, in particular regarding dietary habits. It is of interest to note, that the rise with respect to carcinoma of the colon takes place in the first generation of the migrant population[6].

These observations are a strong indication of external influences acting in the pathogenesis of this and other diseases (carcinoma of the breast and endometrium, cholesterol gallstone disease and cardiovascular disease).

2.1.5. Social class, religion and urbanization

From a number of studies in various countries, it appears that colonic carcinoma is more frequent in persons from higher social classes in contrast to rectal carcinoma which is more prevalent in the lower social classes[7,8]. The influence of religion seems to depend on certain rules regarding life-style. Colorectal cancer is less common among vegetarians, such as the Seventh Day Adventists[9].

Urbanization is associated with a higher incidence, but its influence seems to be variable and is more evident in low incidence countries like Finland[10]. Also, the rise in incidence among migrants from the rural areas to the cities has been regarded as an indication of the relevance of environmental factors for colonic carcinogenesis.

2.1.6. Familial clustering

Clustering of patients with colonic carcinomas at an unusually low age (under fifty) is occasionally noted in certain families. Many of such cases can be classified as hereditary forms of malignancy like the family cancer syndrome or adenomatous polyposis or variants of both[11].

Also, a thorough search into families of unselected patients with colonic carcinoma revealed a greater number of family members with colon cancer than in control families of patients with different diseases[12], but this was not confirmed in a later study[13].

2.1.7. Conclusion

In conclusion, epidemiological data are consistent with a major role of environmental factors in colonic carcinogenesis in man. Much attention has been paid to nutritional aspects, which will be described in more detail, whereas the use of tobacco or laxatives have not been found to be related with colorectal carcinoma[4]. Colon cancer patients have also been questioned about their previous bowel habits. According to this rough estimate, constipation did not seem to be more common than in control subjects[3].

2.2. Aetiology

2.2.1. Predisposing diseases

Important insight into the development of colorectal carcinoma has been obtained from histopathological investigations of benign and malignant epithelial tumours of the colon. Much agreement exists on the concept that the majority of adenomatous lesions in the colon are precursors of colonic carcinoma. In particular, persons with large adenomatous polyps or adenomas with a focus of severe epithelial dysplasia (defined as cytological atypia of the epithelium and changes of the structure of mucosal tubular glands), or with a villous growth pattern, have an increased risk of colon cancer[14]. Also, the simultaneous occurrence of colon carcinoma and large colon adenomas is common.

A recent hypothesis about the interrelationship of environmental and hereditary influences on the so-called adenoma-carcinoma sequence states that the appearance of small adenomas in the large bowel is determined genetically. It is suggested that subsequent growth from small to large size adenomas is controlled by tumour-promoting products in the colon which increase the malignant potential of colonic adenomas and thereby the risk of transition into carcinoma[15].

Another disease predisposing to colon carcinoma development is ulcerative colitis. The precancerous lesions described in this disease are more difficult to recognize during colonoscopy. To assess their malignant potential requires special experience of the pathologist.

2.2.2. Large bowel carcinoma and nutrition

Assessment of human nutrition would ideally entail the weighing of consumed foodstuffs of known composition for large groups of individuals. Since such quantitative measurements are very time-consuming and depend on the cooperation of the population studied, they are rarely used. Instead, virtually all data on nutrition and colon cancer in man are obtained by less accurate methods of limited validity[16] like interviewing. It is therefore not surprising that controversial results have been reported.

The studies on colon cancer and nutrition can be divided into three main categories:

National food consumption data

Such data are being collected from countries all over the world by international agencies like the WHO and FAO. Although corrections can be made for non-human consumption or wastage it is impossible to account for the impact of home farming. It is therefore evident that caution is needed in the interpretation of "correlation studies", which look for associations of incidence or mortality figures of diseases and food consumption data.

Nevertheless, the suggestion that the aetiology of colorectal carcinoma is related to a diet rich in animal fat has been based initially on such data[3,17]. However, figures from some countries are not in accordance with this concept. In Finland, figures of colon cancer mortality are low in spite of a high fat diet. This high fat consumption is due to an extremely high consumption of dairy products; in the rural areas, the daily

intake of milk per head of population is approximately two litres[18]. One explanation appears to be the fibre-rich diet of the Finish population[19].

More recent analysis of international data showed that only the correlation of cholesterol consumption and colon carcinoma mortality remained significant after correction for the impact of other dietary components like total fat, unsaturated fat and dietary fibre consumption[20].

The possible relationship of beef consumption and colonic carcinoma has been indicated by Gregor et al. and was based on WHO data[21]; it is consistent with the rather high colon cancer mortality figures in South American populations with a high meat consumption; this association was not recognized within the United States[22].

Although Burkitt suggested a reciprocal relationship of the dietary fibre intake and the colorectal carcinoma incidence[23], and this may apply to the Scandinavian countries[19], sufficient data in support of this hypothesis are still lacking.

Also, a potential relation of beer consumption and rectal carcinoma has been reported[24,25]; investigations into the causes of death of employees of Danish breweries could not confirm such an association[26].

Retrospective studies

Studies are usually designed as case-control studies. The feeding habits of colorectal cancer patients are compared with respect to dietary constituents or food items with control subjects, employing interview techniques or diaries on food intake. Although these methods may be reproducible, their validity remains questionable especially when enquiries are made into the feeding habits of the more distant past[16].

The results of such studies are variable : some authors did not report any difference in dietary composition[3,27,28], whereas others noted a lower consumption of vegetables (turnips, cauliflower),fruits and vitamins A and C, in particular in patients with rectal carcinoma[29,30]; also, a lower consumption of dietary fibre has been described[31]. It remains to be established whether certain indole compounds, as present in cauliflower and turnips which have proved to be anticarcinogenic in experimental models, also protect against colon carcinoma in man[32,33].

Prospective studies

Data of a longitudinal cohort study on Japanese immigrants in Hawaii as compared to an autochtonous Japanese population group showed a tripling of

the incidence of colon cancer which occurred even in the first generation. The study was initially designed to investigate the role of nutrition in the development of ischemic heart disease, but later data were also collected on cancer and nutrition.

These data, obtained with standardized interview techniques, revealed differences in the feeding habits between the migrated and non-migrated Japanese populations: the former ate more beef and less beans, which is consistentwith a more western diet[34].

2.2.3. Metabolic epidemiology

The old hypothesis that (co)carcinogenic products from bile-acids* and cholesterol may arise from bacterial conversion in the large bowel[35] received renewed interest when epidemiological data showed that an increasing incidence of large bowel cancer is associated with certain dietary habits, in particular a high consumption of animal fat and protein[36]. This was in accordance with the observation that persons from western countries (where colorectal cancer is common) had high faecal concentrations of bacterial bile-salt and cholesterol metabolites and a very predominant anaerobic faecal flora[37,38]. Reports that some species of faecal anaerobic bacteria could be recovered in greater numbers in patients with colon carcinoma have not been confirmed[39,40].

So far no cancer-initiating (complete carcinogens), but only tumour-promoting bacterial bile-salt metabolites have been demonstrated. Also, a role of cholesterol metabolites has been suggested[41], but no consistent evidence has been provided.

The search for carcinogens in faeces has been jeopardized by methodological problems in the use of bacterial mutagenicity tests. Mutagenicity has been used to recognize carcinogens also in biological material. The commonly employed method, the mammalian microsome mutagenicity assay with Salmonella typhi murium strains according to Ames[42] proved to be unreliable due to the toxic growth-inhibiting influence of non-purified faecal extracts on these strains of test bacteria[43,44].

The possibility that some purified mutagenic fractions from stools of healthy persons[45] are indeed related to the development of large bowel carcinoma, is the subject of controlled prospective studies in patients

*Description of biological aspects of bile-acids in general does not allow a distinction between the salt-form and acid-form of this class of steroid compounds. Bile-salt and bile-acid are therefore employed as interchangeable designations in the literature.

with colon adenomas. The purpose of these studies is to measure the effect of ascorbic acid and α-tocopherol supplements or a fibre-rich, fat-depleted diet on the recurrence of new lesions[46]. It has already been shown that such supplements or diets reduce the excretion of certain highly purified mutagenic stool fractions. Whether carcinogenic pyrolysis products, which resemble methylarylamine-compounds and which are formed during the roasting and broiling of meet (browning process) are associated with large bowel carcinoma in man is not clear[47]. Support by epidemiological data is not available.

2.2.4. Animal models of large bowel carcinoma

Induction of colonic carcinoma in rodents has provided the opportunity to test dietary influence in a standardized way on the experimental model. Only certain strains of rats are susceptible to carcinogens with a more or less specific action on the large bowel. The carcinogens commonly used are:

- cycasine derivates, especially 1.2 dimethylhydrazine (DMH) and azoxymethane (AOM). These are powerful carcinogens if administered subcutaneously. Bacterial conversion in the colon enhances the carcinogenic action.
- intra-rectally administered alkyl-nitrosurea compounds like N-methylnitrosoguanidine (MNNG) and methyl-nitrosurea (MNU).
- subcutaneously injected biphenyls like 3.2 dimethyl-4 aminobiphenyl(DMAB) structurally related to mutagenic pyrolysis products of amino-acids (tryptophane).

With these models it has been demonstrated that diets high in fat promote the formation of epithelial tumours in the rat colon[48,49]. This effect has been associated with the enhanced faecal excretion of bacterial bile salt metabolites, which have been shown to promote the induction of colon carcinomas and adenomas by MNNG in the rat[50].

It is less certain whether the consumption of poly-unsaturated fats favours the development of large bowel cancer in man[51] as has been shown in certain circumstances in rats[52]. The cocarcinogenic effect of polyunsaturated fats has been ascribed to be a change in the phospholipid composition of cell membranes as shown in liver cells[53], but it remains to be established for colonic epithelial cells.

One should be cautious of extrapolating the role of bile-salt metabolites in colonic carcinogenesis from the animal model to the human situation, especially because the underlying mechanism of tumour-promotion by bile-salts is not well understood. However, it seems to be in accordance with many epidemiological data.

The high enzymatic activities (like β-glucuronidase activity) in the stools of rats on a protein and fat enriched diet are also in agreement with a role of the colonic flora in initiation or promotion[54].

Further, a cocarcinogenic effect has been attributed to cholesterol (or its metabolites) causing a raised number of induced large bowel tumours when supplemented to the rat diet[55], but these findings could not be confirmed[56].

A protective action of dietary fibre against the development of colon carcinoma as has been suggested[23], has been associated with the adsorbing and diluting effect on (co)-carcinogenic compounds in the colon. Also, a reduction in the activity of bacterial enzymes due to acidification of the colonic contents by formation of volatile fatty acids from dietary fibre has been noted[57].

Using high doses of bran rich in hemicellulose, it has been possible to demonstrate a reduction in the number of A.O.M. induced colon tumours during a low fat diet in the rat[58]. However, studies on the preventive effect of dietary fibre in experimental colonic carcinogenesis often produce controversial results. Poorly standardized composition of dietary fibre supplemented rat food, variable study designs and different schedules of carcinogen administration may be responsible.

A lack of effect of dietary manipulations after the phase of carcinogen administration is consistent with a promoting and not with an initiating effect of dietary factors on experimental colonic carcinogenesis[59]. The antagonism of colon carcinogenesis by antioxidative compounds has been poorly investigated. Disulfiram and ascorbic acid prevented the carcinogenic effect of DMH administration in the colon[32].

2.2.5. Conclusion

In conlusion, experimental colorectal carcinogenesis can be influenced by dietary factors; little is still known in this respect about colonic

carcinogenesis in man.

It is of interest to note that a high fat diet (4 to 6 weeks) raises faecal bile-acid excretion without alteration of intestinal transit time or faecal flora[60]. An increment of the (dietary) fibre content causes higher stool weights and usually a more rapid intestinal transit; the effect on faecal bile-salt excretion and colonic bile-salt metabolism depends both on the form of the dietary fibre source (fibre-rich diet or fibre-supplemented diet, cooked or uncooked, particle size) and on individual factors (age and pre-existent bile-acid metabolism)[59,62,63,64]. This may account for the different results which have been reported[62]. Diet-intervention studies in persons with an increased risk of colon carcinoma (adenoma patients) are needed to answer the question whether, also in man, a reduction in tumour-promoting properties of the intra-colonic environment can be achieved by changes in diet.

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CHAPTER III

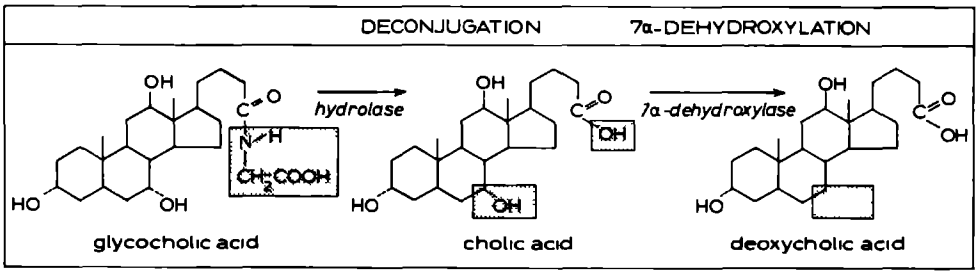
QUANTIFICATION OF BILE-SALT METABOLISM AND INTESTINAL TRANSIT TIME

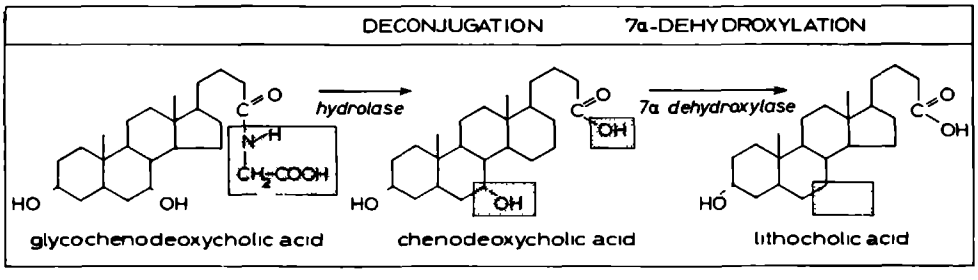
3.1. Formation and biological effects of bile-acids

Bile-acids are secreted into the bile canaliculi after hepatic synthesis of their steroid moiety from cholesterol and after conjugation with taurine or glycine[1,2]. The majority of the secreted bile-acids are, however, not newly synthesized but extracted from the portal blood by an active uptake mechanism over the sinusoidal liver cell membrane. Only negligible amounts of sulphated or glucuronidated bile-acids can be demonstrated in bile of healthy subjects[3,4].

Bile-acids have various physiological functions such as stimulation of bile flow[5], micellar solubilization of cholesterol[6], facilitation of intestinal digestion and fat absorption[7,8]. The large bowel is in normal subjects the only site of bacterial transformation of bile-acids synthesized in the liver (primary bile-acids) into conversion products (secondary bile-acids). Deoxycholic and lithocholic acid are the principal secondary bile-acids in human bile derived from cholic and chenodeoxycholic acid respectively[9] (Fig.1).

Figure 1 : Principal transformations by bacterial enzymes of the primary bile-acids in man.





Particularly deoxycholate exerts important effects on colonic functions such as an increase in epithelial cell proliferation[10], motility[11] and water and electrolyte secretion[12]. Finally, faecal bile-salt excretion constitutes a major excretory pathway of cholesterol from the body[13].

3.2. Enterohepatic circulation of bile-acids

A prominent biological feature of bile-acids is their extensive enterohepatic circulation. After absorption by the small intestinal mucosa, facilitated (in the ileum especially) by an active transport mechanism, bile-acids are drained into the portal blood and then efficiently taken up by the liver and secreted into the biliary tract.

Driving forces of the enterohepatic circulation are activated during a meal by the release of cholecystokinin from the small intestine causing gallbladder contraction[14], hepatic bile secretion[15] and increased bile duct and intestinal motility and relaxation of the sphincter of Oddi[16]. In the fasting state, enterohepatic bile-acid circulation is reduced to a lower level and appears to be determined by interdigestive sphincter of Oddi and gallbladder activity controlled by gut hormones such as motilin [17,18].

The proportion of bile-acids which is not absorbed by the small intestine (2-5%) per cycle amounts to an average loss of 30% of the primary bile-acid pool with six to twelve enterohepatic circulations per day. In the colon, rapid deconjugation and subsequent 7α-dehydroxylation by bacterial enzymes occurs leading to the formation of the major faecal bile-salts, lithocholate from chenodeoxycholate and deoxycholate from cholate[19]. Also variable amounts of keto- and iso- bile-acids can be formed by dehydrogenation and epimerization of hydroxyl groups. Further aromatization of bile-acids has been thought possible, but identification of such conversion products is complicated[20,21].

Colonic absorption of cholic acid, deoxycholic acid and chenodeoxycholic acid is well established. Lithocholate also appears to be absorbed to some extent. However, direct evidence is lacking because lithocholate is poorly water soluble which precludes perfusion experiments to test intestinal absorption[22,23,24].

Conjugates of ursodeoxycholate, the 7β-epimer of chenodeoxycholic acid, and lithocholate, both present in bile in minute amounts are of colonic origin. Rapid sulphation of lithocholate in the liver decreases intestinal reabsorption causing its small pool size[25,26]. Sulphation also protects against the hepatotoxicity of lithocholate[27]. The possibility of hepatic synthesis of ursodeoxycholic acid from 7-keto-lithocholic acid, a colonic bacterial metabolite of chenodeoxycholate absorbed from the gut, has recently been disputed[28].

Deoxycholate, the major secondary bile-salt in bile (up to 45%) resembles chenodeoxycholate with respect to its behaviour within the enterohepatic circulation. Both bile-salts carry two hydroxyl groups (Fig.1) and are, in contrast to lithocholic acid, present in bile in almost unsulphated form.

Enterohepatic circulation rates of individual bile-acid species further depend on intestinal absorption and hepatic extraction. Clearance of dihydroxylated bile-acids or of their conjugates from the portal flow is less efficient than that of cholic acid or cholic acid conjugates respectively[29]. Intestinal absorption is determined by physico-chemical properties associated with steric structure and ionic charge (characterized by pKa values, see Table I).

Table I MODE OF CONJUGATION AND pKa VALUES OF BILE-ACIDS

	approximate pKa
Unconjugated	6
Glycine conjugation	4
Taurine conjugation	2

In the jejunum some absorption of glycine conjugated (25%) and considerable absorption of unconjugated (60%) dihydroxylated bile-acids occur[30]. A saturable active transport of conjugated bile-acids and unconjugated cholic acid has been demonstrated in the ileum, playing a crucial role in bile-acid conservation within the enterohepatic circulation[31].

In general, dihydroxylated bile-acids are assumed to have a shorter enterohepatic circulation than trihydroxy bile-acids.

This is probably due to a difference in site of preferential absorption from the intestine. Serial measurements of post prandial serum bile-acids have confirmed this view[32]. For similar reasons it is assumed that glycine conjugates have a more rapid enterohepatic circulation than taurine conjugates of the same bile-acid class.

3.3. Determination of bile-salt metabolism in bile

The preparation and application of ^{14}C and ^{3}H labelled steroid compounds in human studies have allowed the determination of hitherto undiscovered aspects of the metabolism of endogenous substances like cholesterol and bile-acids in man[33,34,35].

Lindstedt, using a so-called isotope dilution technique in bile, according to the volume distribution principle, demonstrated in 1957 that decay of specific activity (desintegrations per minute per quantity of bile-acids) of cholic acid in human bile after oral administration of ^{14}C-labelled cholic acid was semi-logarithmic. His calculations were based on analyses of duodenal bile samples collected on five to seven consecutive mornings (duodenal bile is a descriptive term for bile-rich duodenal contents as obtained by duodenal intubation and subsequent stimulation of the gallbladder bile flow with cholecystokinin). The turnover of the cholic acid pool in man could thus be described by a first order kinetic model, which is consistent with the physiological concept of the enterohepatic bile-acid circulation as one single compartment. He calculated cholic acid pool size from the ratio of the administered dose of radioactivity and the value of specific activity obtained by extrapolation of the semilogarithmic specific activity decay curve to the time of administration (Fig 2). The decline of specific activity in bile per 24 hours also indicates the fraction of the cholic acid pool which leaves the enterohepatic circulation per day and which is called the daily fractional turnover rate(k). The half-life($T_{\frac{1}{2}}$) of the cholic acid pool can be derived from k by the following formula :

$T_{\frac{1}{2}} = \ln 2 / k.$ [34,35]

Validity of the method requires a complete mixing of the administered bile-acid label with the circulating bile-acid pool, sufficient stability

of the radioactive label under physiological conditions and a steady state of the enterohepatic circulation, in which a constant fraction of the bile-acid pool lost from the enterohepatic compartment (fractional turnover) is replaced by an equal amount of newly synthesized bile-acid.

First order kinetics have also been shown for chenodeoxycholic acid and for deoxycholate, the major bacterial bile-salt metabolite in human bile[35]. Synthesis rates of primary bile-acids in man can be calculated by multiplication of their pool sizes by their fractional turnover rates (Fig 2).

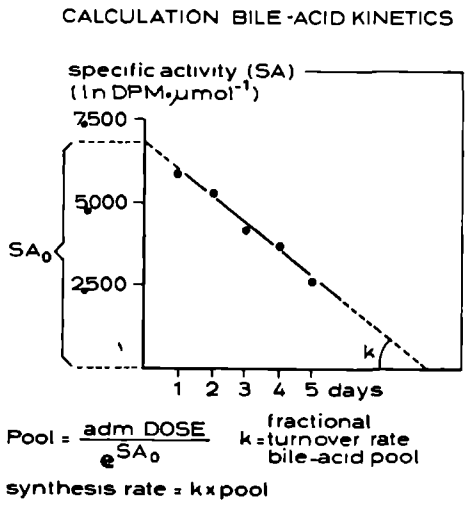


Figure 2 :

Determination of the kinetic parameters of bile-acid metabolism by isotope dilution in bile ; SA = logarithm of specific activity.

The proportion of deoxycholate in bile of healthy subjects varies considerably (2 to 45%) with an average of approximately 45%. The daily input of deoxycholate into the circulating bile-acid pool is chiefly determined by the amount of newly-formed deoxycholate, the major bacterial metabolite of cholate (Figs 1 and 3), but possibly also by other factors like colonic pH and intestinal transit time which will be described in chapter IV.

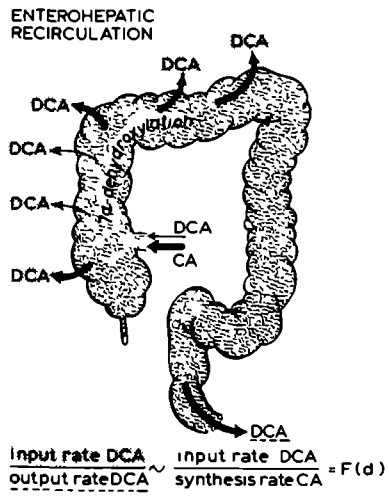


Figure 3 :
Schematic representation of deoxycholate (DCA) and cholate (CA) metabolism in the large bowel.
F(d) = 7 α dehydroxylation fraction.

The enterohepatic turnover rate of lithocholate is high and does not follow first order kinetics due to a marked suphation rate in the liver[26]. Colonic absorption of this monohydroxy bile-salt with a relatively low polarity is less than that of deoxycholate. This can be explained by the intracolonic environment favouring adsorption to bacteria and precipitation.

Daily absorption of deoxycholate from the colon (as indicated by its input rate into the circulating bile-acid pool) and synthesis rates of primary bile-acids are assessed in the same way using isotope dilution methods in bile. Multiplication of deoxycholate pool size by the fractional turnover rate of deoxycholate yields the absorption rate of deoxycholate from the large bowel (Fig 2). Dual isotopic bile-salt investigation in bile permits simultaneous determination of cholate and deoxycholate metabolism after satisfactory separation and purification by thin layer chromatography of cholate and deoxycholate fractions of a hydrolyzed extract of bile-acids from bile[36].

Biliary bile-acids can be measured by colorimetric or enzymatic methods [37,38], but gas-liquid or high-pressure liquid chromatography offers the advantage of a higher specificity[39].

For measurement of radioactivity of ^{3}H or ^{14}C isotopes, standard liquid scintillation counting techniques with external quench correction can be applied[40].

The dual isotope dilution method using ^{14}C-deoxycholate and ^{3}H-cholic acid allows an estimate of the efficiency of colonic deoxycholate absorption as may be obtained from the ratio of colonic input of deoxycholate into the bile-acid pool and of cholic acid synthesis. The latter matches the faecal excretion of cholic acid metabolites (mainly deoxycholate, Fig.3). This ratio has been called the 7α-dehydroxylation fraction (F_d) by Hofmann[35] and has an average of 30% ranging from 2 to 80% in normal subjects[41].

The reproducibility of the dual isotope dilution method employed in our present studies can be shown by duplicate investigations already done on three subjects (Table II).

TABLE II
VARIATION OF CHOLIC ACID SYNTHESIS RATE AND DEOXYCHOLATE INPUT RATE IN 3 YOUNG HEALTHY MALES DETERMINED ON TWO OCCASIONS ($\mu mol.Kg^{-1}.d^{-1}$)

Subject no.	Deoxycholate input rate		Cholic acid synthesis rate	
1	2.4	2.5	8.9	7.8
2	3.9	3.8	20.1	18.5
3	1.1	1.4	7.9	5.6

A detailed description of the analytical techniques is given later on in Chapter IV.

Bile-acid metabolism of both primary bile-acids may be determined simultaneously from one bile sample using a dual isotope dilution technique. The bile-salt labels have to be administered on two consecutive days prior to bile sampling[42].

Most investigators, however, prefer the classical method described by Lindstedt, which requires at least four to five collections of duodenal bile[35]. The main drawback of the one sample method is that the presence of a steady state bile-acid metabolism cannot be confirmed by a semilogarithmic decay of specific activity. Moreover, simultaneous determination of cholic acid and deoxycholate metabolism is impossible using this approach because of the precursor-product relationship of cholic acid and deoxycholate.

Apart from a semilogarithmic specific activity decay curve as judged from the linear regression coefficient, a stable biliary bile-acid composition is also a reliable indicator of a steady state of the enterohepatic bile-acid circulation requiring also multiple bile sample collections.

3.4. Definition of intestinal transit time

Intestinal transit time can be regarded as the interval between oral ingestion of solids or fluids and faecal excretion of their non-absorbable and non-digestible residues and is referred to as mouth-anus transit time. Since passage through the first part of the digestive tract proximal to the colon usually takes less than two or three hours in healthy subjects [43], colonic transit will be its major determinant.

3.5. Measurement of intestinal transit time

To quantify intestinal transit time, non-absorbable markers of various consistencies (fluid or solid), shapes, colours and numbers and various modes of administration have been used (all in one dose or in several separate doses per day over a period of time)[44,45,46,47,48,49]. The choice depends on the question to be answered. If one wishes to know whether alterations in the dietary composition over a long period are associated with a change in transit time, administration of solid markers in daily separated doses will be preferred to reduce the effect of temporary fluctuations in faecal flow[50]. This time-consuming method has been modified by using markers of various shapes in one subject which allows determination of the mean intestinal transit time from one single stool[51].

In an outpatient setting, however, application of these continuous marker techniques requires understanding and cooperation of the investigated subject, both with respect to ingestion of the markers as to accurate stool collection. Another application of continuous marker techniques is the calculation of corrections for the irregularity of faecal excretion of lipids and steroids in metabolic studies[45,52].

A method using ingestion of all markers in one dose, as described by Hinton et al[49] is, however, easier to supervise and more reliable regarding the completeness of the intake of the markers. It will give information concerning actual intestinal transit in the period immediately after ingestion rather than provide an average figure. Excretion of 80% of the markers is used as a yardstick for intestinal transit time since the last 20% of markers may be delayed disproportionately.

The use of $^{51}CrCl_3$ dissolved in water as non-absorbable marker and administered as one oral dose, offers the additional advantage that completeness of stool collection by the investigated subject at home can be confirmed by determination of the retention of radioactivity with a whole body counter[41].

It should be clear from the definitions that the results of measuring transit time as mean intestinal transit time and according to the method of Hinton et al. may differ. In short term studies of bile-acid metabolism, the method described by Hinton appears to be the most appropriate one for simultaneous measurement of intestinal transit time during the study period. We also applied this method to our study subjects using labelled chromium chloride as a liquid marker and pellets cut from polyvinyl tubing as solid markers. It was anticipated that various forms of non-absorbable markers may yield non-identical results[41,53]. It has been suggested that radio-opaque pellets travel through the intestine as markers of the solid stool phase whereas soluble substances like $^{51}CrCl_3$ are better indicators for the liquid phase. The use of radio-opaque pellets may therefore be preferable for assessment of transit time in association with studies of bile-salt metabolism, since bile-salts are well adsorbed to solid faecal material designated as dietary fibre[53].

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CHAPTER IV

Age dependent differences in human bile acid metabolism and 7α-dehydroxylation

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Abstract. It has been suggested that transformation of secondary bile acids into (co)carcinogenic compounds may have a role in the development of cancer of the large bowel. Because of age dependent differences of this disease we undertook a study of cholic and deoxycholic acid metabolism of eleven young adults (group A, 20–30 years old) and eleven elderly persons (group B, 55–75 years old) with a double isotope dilution method. Daily food intake was standardized individually and gut transit time measured with radio-opaque pellets and labelled chromium chloride.

The 7α-dehydroxylation fractions (the ratio of deoxycholic acid input rate from the large bowel to cholic acid synthesis rate) were higher in group B ($P < 0{\cdot}01$) due to higher deoxycholic acid input rates ($P < 0{\cdot}005$), especially when individuals from both groups with rapid gut transit were compared. As contributory factor was recognized the higher fractional turnover rate of cholic acid in group B. Pool sizes and synthesis rates of cholic acid and gut transit times were similar. In group A, but not in B, gut transit times correlated with deoxycholic acid input rates ($P < 0{\cdot}01$).

The differences in bile acid metabolism may be related to a more effective colonic absorption of

deoxycholic acid in the elderly persons with a concomitant decrease of active ileal absorption of cholic acid in the elderly persons. Differences in diet or gut transit time between both groups do not seem to be the underlying mechanism.

Key words. Age, bile acid metabolism, colonic carcinoma.

Introduction

Colorectal cancer is a disease of individuals of advanced age in Western countries [1]. Transformation of bile acids by colonic bacteria into carcinogenic or cocarcinogenic compounds has been implicated as a possible cause [2, 3]. This concept arises from faecal bile acid studies and epidemiological data. High consumption of animal fat has been observed in countries with high incidence of colorectal cancer [4]. This consumption enhances faecal bile acid excretion in man [5]. Accelerated intestinal transit due to a high fibre intake is thought to protect the colon from exposure to bile acid degradation products [6].

Cholic acid (CA) is a major primary bile acid in man. After leaving the enterohepatic circulation it is deconjugated and almost completely converted in the large intestine into its secondary bile acid deoxycholic acid (DCA). Not all DCA is excreted subsequently with the faeces. A fraction is absorbed by the large intestine and carried back into the enterohepatic circulation. Absorption of this fraction might indicate the degree of exposure of the large intestinal mucosa to other bile acid metabolites crossing the colonic wall like DCA.

It would be of great value to know whether colonic bile acid absorption is related to colorectal cancer or to

an increased risk for this disease present in patients with adenomatous polyps, polyposis coli and ulcerative colitis.

Application of a double isotope dilution method using sodium [24-^{14}C]deoxycholate and [2,4-^{3}H]cholic acid allows simultaneous determination of CA- and DCA-metabolism (pool sizes, fractional turnover rates, CA-synthesis rate and DCA-input rate) [7]. From the latter two the 7α-dehydroxylation fraction (F(D)) can be calculated as the ratio of DCA-input rate of CA-synthesis rate [8]. This approximates to the ratio of absorbed and excreted DCA, provided that almost all CA in the large intestine undergoes 7α-dehydroxylation.

A preliminary study in eleven young adults (group A) and eleven elderly persons (group B) was performed to assess the influence of age, gut transit time and dietary composition on CA- and DCA-metabolism.

Materials and Methods

Subjects. Detailed data of all participating healthy volunteers are presented in Table 1. Persons with the following were excluded from the study: individuals who were subjected to cholecystectomy or major intestinal surgery in the past; those with known liver, gallbladder or intestinal disease, severe hyperlipoproteinaemia, diabetes mellitus and patients using drugs, especially antibiotics, laxatives and hypnotics.

Alcohol consumption not exceeding 10 g daily was allowed. Body weight was recorded thrice during the study and proved to be stable. It was relatively less in young adults than in the older persons as judged from the deviation from standard body weight.

Physical examination, tests for glucose and protein

Table 1. Data on investigated subjects belonging to two different age groups

	Group A	Group B	*P*
No. of subjects	11	11	
Age*	21·8 ± 1·8	67·3 ± 4·5	
Sex ratio (M/F)	5/6	6/5	
Weight* (kg)	67·6 ± 5·1	69·1 ± 12·3	N.S.
Δ Ideal weight* (%)	−13 ± 8	+11 ± 17	<0·0005
Serum triglyceride* (mmol/l)	1·03 ± 0·41	1·56 ± 0·73	
Serum cholesterol* (mmol/l)	4·6 ± 1·0	5·4 ± 1·0	<0·05

N.S. not significant. * Mean ± SD.

$$\%_0 \text{ of Ideal weight: } \frac{\text{weight (kg)}}{\text{length (cm)} - 100} \times 100\ (\%_0).$$

in the urine and blood analysis for erythrocyte sedimentation rate, liver and renal function and fasting serum lipids did not reveal abnormalities apart from slightly elevated serum triglyceride levels (3·0 and 2·3 mmol/l) in two elderly persons. Stools of all were guiac negative and did not contain intestinal parasites. Normal gallbladder function was ascertained by obtaining dark brown bile after administration of cholecystokinin or its synthetic C-terminal octapeptide sincalide. Oral cholecystograms were obtained, when there was any suspicion of cholelithiasis.

Before their participation in the study all gave informed written consent.

Experimental design. None of the subjects was hospitalized during the study. Average daily food intake was defined from interview data by the same dietician. In their homes all participants checked the accuracy of this estimate using written instructions and

weighing all foodstuffs before consumption. After the necessary adjustments they adhered to this individual schedule from 1 week before till the end of the study in order to maintain a steady state. Ranges and means $\pm$ SD of the dietary constituents in both groups are shown in Table 2 as calculated from Dutch food tables [9].

To study bile acid metabolism 40 μCi [2,4-^{3}H]cholic acid and 10 μCi [24-^{14}C]sodium deoxycholate (Radiochemical Centre, Amersham, England) were injected intravenously. Both substances were more than 97% pure according to thin layer chromatography. On the next five mornings 2 ml fasting duodenal bile samples were collected after gallbladder stimulation with cholecystokinin (Karolinska Institute, Sweden) or sincalide (Kinevac, Squibb, Princeton, N.Y., U.S.A.).

To define gut transit time 5 μCi $^{51}CrCl_3$ (Radiochemical Centre, Amersham) in distilled water and twenty-five radio-opaque pellets were ingested after the withdrawal of the first bile sample. The pellets were prepared by cutting 3 mm segments from polythene tubing with a radio-opaque marker, external diameter 4·5 mm, weight 20 mg, specific gravity 1·20 (Suction catheter Argyle, Brunswick Co., Belgium) [10]. The estimated radiation dose of the gallbladder as critical organ in this bile acid kinetic study is 0·8 rad, in the transit time measurement with $^{51}CrCl_3$ the critical organ is the rectosigmoid receiving 0·010 rad approximately.

Analyses. All bile samples were stored at $-20°C$ immediately after collection until analysis. 0·5 ml bile was deconjugated by incubation overnight at 37°C with cholyl glycine hydrolase (E.C.3.5.24., Sigma Chemical Co., St Louis, Mo., U.S.A.).

Table 2. Dietary constituents of the food intake in young adults group A) and in elderly persons (group B) in g day^{-1} as mean ± SD and their ranges

	Group A	Group B	*P*
Total proteins	94 ± 27 (51–139)	75 ± 15 (54–104)	$P < 0{\cdot}05$
Animal protein	64 ± 19 (31–90)	56 ± 12 (37–81)	N.S.
Vegetable proteins	30 ± 10 (18–49)	19 ± 4 (10–24)	$< 0{\cdot}001$
Total fat	80 ± 25 (47–134)	79 ± 10 (58–93)	N.S.
Animal fat	63 ± 26 (35–117)	63 ± 13 (49–79)	N.S.
Vegetable fat	18 ± 12 (5–48)	16 ± 14 (3–44)	N.S.
Cholesterol	*218 ± 68 (140 ± 356)	*211 ± 57 (145–343)	N.S.
Saturated + monounsaturated fat	68 ± 19 (38–109)	64 ± 8 (51–78)	N.S.
Polyunsaturated fat	12 ± 5 (5–21)	13 ± 9 (3–30)	N.S.
Carbohydrates	307 ± 75 (202–421)	200 ± 46 (129–249)	$< 0{\cdot}005$
Dietary fibre	36 ± 10 (23–55)	22 ± 5 (12–29)	$< 0{\cdot}001$

N.S. not significant.
* mg day^{-1}.

Following acidification with 0·5 ml concentrated hydrochloric acid bile acids were extracted thrice with diethylether. After evaporation under a nitrogen stream until dryness the extract was dissolved in 0·5 ml methanol and used for determination of bile acid kinetics and biliary bile acid composition using established methods of thin layer adsorption chromatography [11], gas–liquid chromatography [12] and liquid scintillation counting as reported previously from our laboratory [13]. Pool sizes and fractional turnover rates of CA and DCA, CA-synthesis rates and DCA-input rates could be calculated from CA- and DCA-specific activity decay curves as outlined by Lindstedt [7] and Hofmann [8]. The 7α dehydroxylation fractions, F(D), were derived from DCA-input rates and CA-synthesis rates and indicate the ratio of absorbed to excreted DCA [8]. The linear correlation coefficients of the semilogarithmically plotted decay curves averaged 0·99 ± 0·01 (mean ± SD) for DCA and CA.

Total bile acid pool sizes were derived from DCA-

plus CA-pool size according to their percentual contribution to the total bile acid concentration, which was defined as the sum of CA, DCA, chenodeoxycholic (CDCA) and lithocholic acid (LCA). Serum cholesterol was measured according to the method described by Röschlau [14], serum triglycerides according to the method of Demacker [15]

Determination of gut transit time. Gut transit time was defined as the period between ingestion and excretion of 80% of the markers [10]. Each stool was collected in separate freezer bags and kept in dry ice the next 4 days following ingestion of the markers. After radiography of the bags the stools were homogenized with equal amounts of distilled water for measurement of $^{51}CrCl_3$. As has been described previously, completeness of stool collection could be verified by daily measurement of the retained $^{51}CrCl_3$ with a total body counter [16].

Statistics. Data of both groups were evaluated with the Wilcoxon's rank sum test with confidence level at 0·05. Linear regressions have been calculated by the method of least squares, their significance was obtained from Pearson's correlation coefficient *r*.

Results

Bile acid kinetics

Individual fractional turnover rates of CA and DCA, CA-synthesis rates, DCA-input rates and (F)D values are shown with their medians in Figs. 1 and 2 for both age groups, whereas medians of pool sizes and

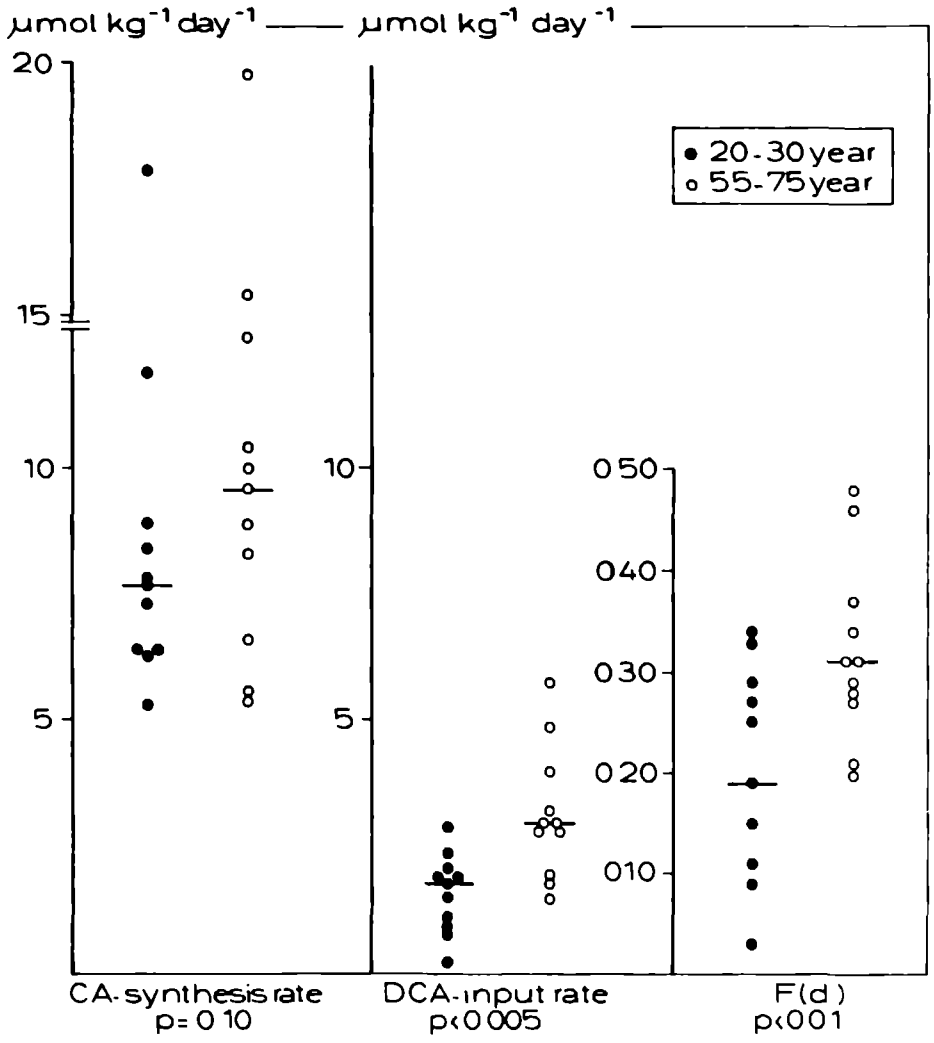

Figure 1. Cholic acid synthesis rates (left panel), deoxycholic acid input rates (middle panel) and 7α-dehydroxylation fractions (right panel) in young adults (•) and elderly persons (o). CA: cholic acid; DCA: deoxycholic acid; F(D): 7α-dehydroxylation fraction.

their ranges are given in Table 3.

Pool sizes of DCA derived from total bile acid pools (determined by means of [2,4-^{3}H]cholic acid in combination with [24-^{14}C]deoxycholic acid kinetics) and from DCA percentages in the biliary bile acid composition were not different from those measured by

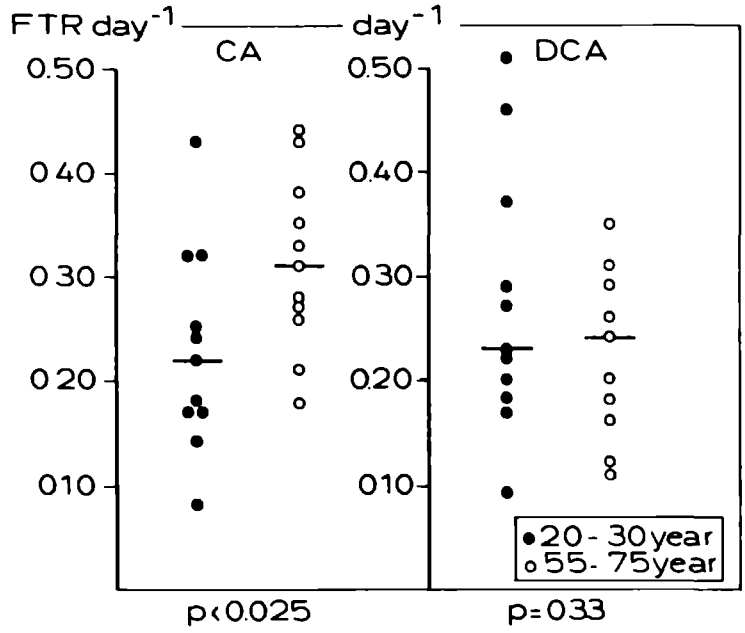

Figure 2. Fractional turnover rates of cholic acid (left panel) and deoxycholic acid (right panel) in young adults (•) and elderly persons (o). CA: cholic acid; DCA: deoxycholic acid; F.T.R.: fractional turnover rate.

[24-^{14}C]deoxycholic acid kinetics only ($P > 0.05$). Both showed a good linear correlation (Fig. 3).

The fractional turnover rates of CA were higher in elderly persons than in young adults (median 0·22 day^{-1}) for group A as against 0·31 day^{-1} in group B

Table 3. Medians and ranges of cholic acid (CA), deoxycholic acid (DCA) and total bile acid pools of eleven young adults (group A) and eleven elderly persons (group B) in μmol kg^{-1}

	Group A	Group B	*P*
CA pool	37·3 (19·6–91·7)	31·0 (16·9–53·9)	N.S.
DCA pool	7·6 (0·5–17·9)	13·4 (5·9–36·5)	<0·005
Total pool	74·0 (40·6–153·3)	88·5 (50·6 122·6)	N.S.

N.S. not significant.

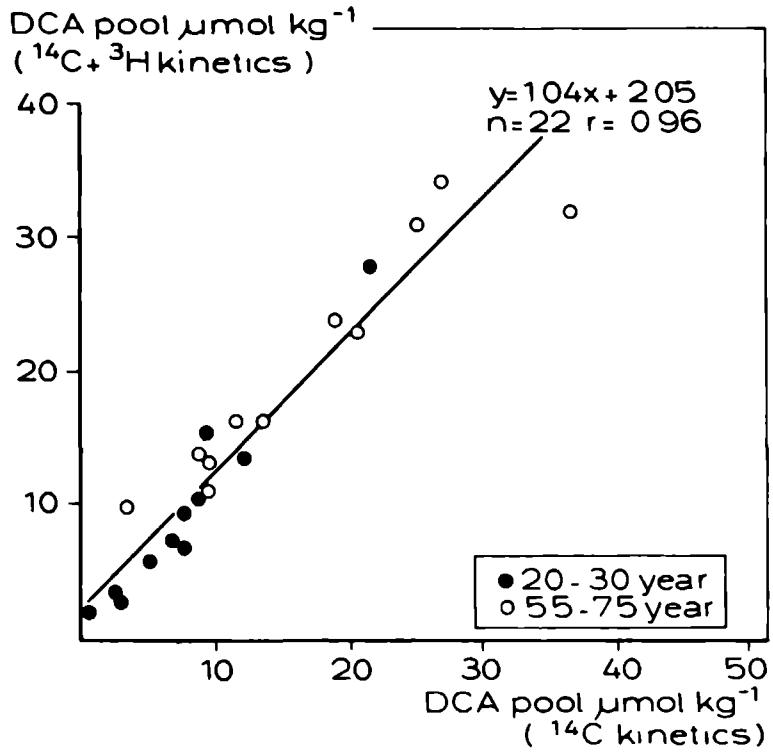

Figure 3. Deoxycholic acid pool sizes derived from total bile acid pools (determined by means of [2,4-^{3}H]cholic acid- and [24-^{14}C]deoxycholic acid kinetics) and from percentages of DCA in the biliary bile acid composition as compared to deoxycholic acid pool sizes derived from [24-^{14}C]deoxycholic acid kinetics in young adults (•) and elderly persons (○). CA: cholic acid; DCA: deoxycholic acid.

(Fig. 1). The median of CA-pool sizes was lower in group B (31·0 versus 37·3 μmol kg^{-1}) (Table 3), but differences between both groups in this respect were not statistically significant. The median value of CA-synthesis rates was slightly lower in young adults (7·6 compared to 9·6 μmol kg^{-1} day^{-1} in groups A and B), but the differences between both groups also did not show statistical significance (Fig. 2). Low DCA-input rates and DCA-pool sizes in four young adults caused significant differences of these parameters

between both groups. This is reflected by higher F(D) figures in group B (median 0·31 in group B and 0·19 in group A) (Fig. 2).

If gut transit time is taken into account, it appears that, in subjects with an intestinal transit less than 3 days (65 h), DCA-input rates of elderly persons exceeded those of young adults (Fig. 4) ($P < 0{\cdot}0025$). This was less obvious for subjects with intestinal transits beyond 65 h.

Gut transit time

Measurement with $^{51}CrCl_3$ showed a slightly shorter intestinal transit in the elderly than in the young subjects. Such a difference between both groups was not found for the transit times defined with radio-opaque pellets (Fig. 5). A correlation of the intestinal transit to the DCA-input rate proved to be present in group A ($n = 11$, $r = 0{\cdot}81$ $P < 0{\cdot}01$), but not in group B using the pellet method. A similar correlation was not found with the $CrCl_3$ method.

Dietary composition

The average daily food intake in both groups is shown in Table 2. Intake of vegetable protein, carbohydrates and dietary fibre of group A exceeded that of group B.

The highest consumption of dietary fibre (37–55 g day^{-1}) was recorded in those four young adults who showed the lowest DCA input rates and shortest gut transit times (about 25 h only). Nevertheless, there was no correlation between any of the dietary constituents mentioned above and DCA input rates.

An inverse relation of the daily intake of animal

protein and cholesterol to gut transit time was recorded in young adults ($r = -0{\cdot}79$ resp. $-0{\cdot}62$), but not in elderly persons ($r = +0{\cdot}17$ resp. $-0{\cdot}21$). There were no correlations of animal fat, dietary fibre or any other dietary component to gut transit time.

Discussion

Bile acid metabolism

Values of CA-kinetic parameters in group B are in agreement with findings in control subjects of Einarsson *et al.* [17], who used [24-^{14}C]cholic acid.

We carried out bodyweight correction on our results according to the recommendations of Hofmann & Hoffman [8], since we support the concept that bile

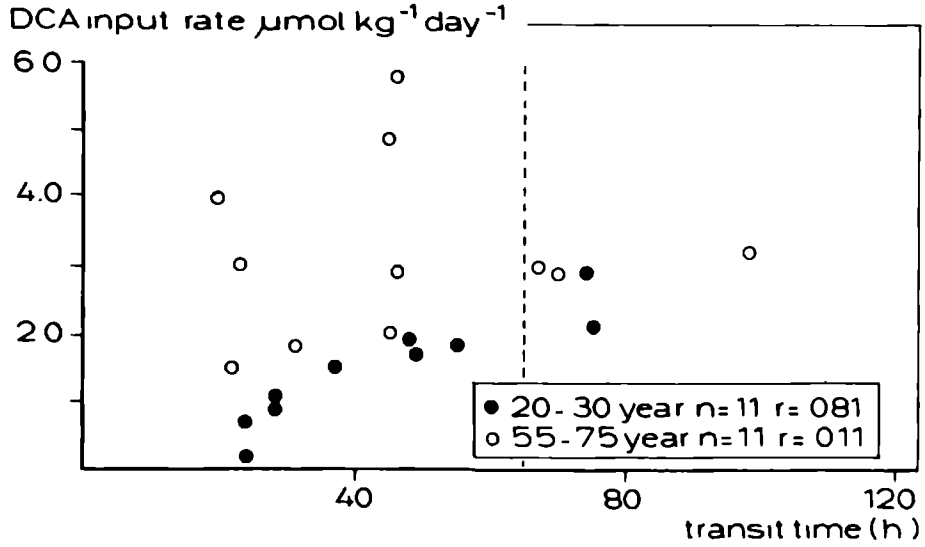

Figure 4. Scatter diagram of deoxycholic acid input rate and gut transit time in young adults (•) and elderly persons (o). All subjects at the left side of the interrupted line have intestinal transit times of less than 3 days (65 h). DCA: deoxycholic acid.

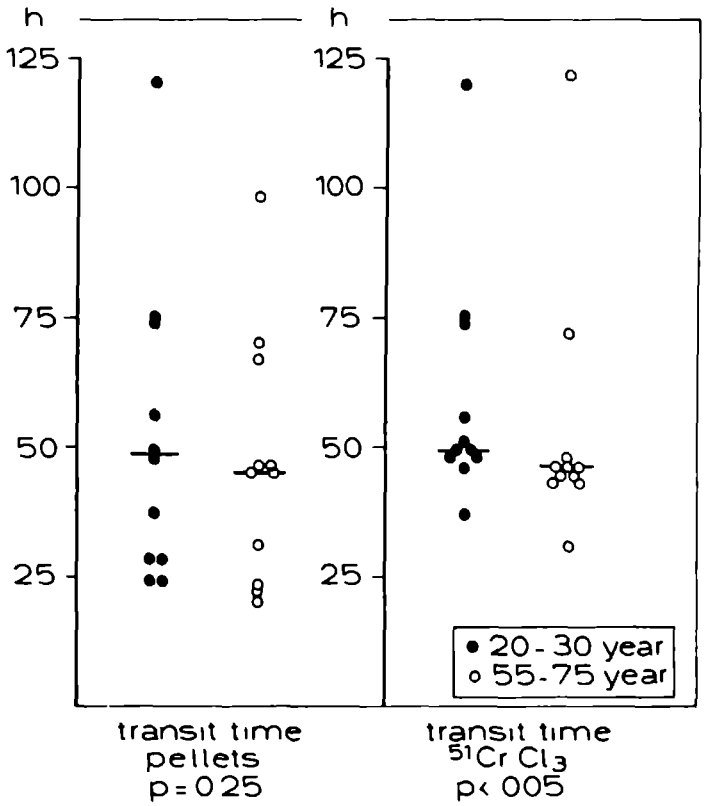

Figure 5. Gut transit times in young adults (•) and elderly persons, measured with radio-opaque pellets (left panel) and $^{51}CrCl_3$ (right panel).

acid synthesis should be related to hepatic weight and therefore also to actual bodyweight.

To assess non-specific loss of tritium from [2,4-^{3}H]cholic acid [18] we investigated the relation of DCA-pool size derived from ^{14}C-kinetics to pool size obtained as a fraction of the total bile acid pool size. Total pool size calculations are always indirect and rely on the assumption that individual biliary bile acid composition reflects the pool sizes of the various bile acids. It has been shown, however, that with this approach CA-pool size will be overestimated and CDCA- and DCA-pool size underestimated due to

lower enterohepatic cycling frequencies of CA as compared to CDCA and probably also DCA [19]. To minimize this problem we preferred to use the sum of CA- and DCA-pool size derived from ^{3}H-resp. ^{14}C-kinetics for indirect calculation of DCA-pool sizes.

As can be noted in Fig. 3, indirectly obtained pool sizes are still about 2 μmol kg^{-1} higher. This error seems to be due to the aspecific tritium loss since it compares well with the urinary tritium excretion (7–9% of the administered dose in our subjects).

Comparison of DCA pool sizes in both groups will not be invalidated by such a relatively small systematic error especially because no significant difference in cholic acid pool size appeared to be present (Table 3).

For DCA-input rate, F(D) and CA-fractional turnover rate differences were recorded between young adults and elderly persons (Figs. 1 and 2), especially in those with a rapid intestinal transit (Fig. 4).

The higher F(D) in group B is mainly caused by a higher DCA-input rate and not by a lower CA-synthesis rate, which in fact tended to be higher in the elderly (Fig. 1). An increased availability of CA in the large bowel, as appears from the higher CA-fractional turnover rates, might to a certain extent account for this higher colonic absorption of DCA in group B. A similar difference of CA-fractional turnover rate was found by Valdevieso *et al.* [20] in young and elderly Chilean women, but did not reach a significant level in their study, probably due to the small number of subjects investigated.

The higher CA-fractional turnover rate in our study does not seem to be associated with a higher cycling frequency of bile acid circulation, since total bile acid pools in group A and B were similar (Table 3). As has been shown by Northfield *et al.* [21], total bile acid pool

and cycling frequency are inversely related. Therefore the increase of CA-fractional turnover rate in elderly persons is more likely due to a decrease of active ideal CA-absorption.

Regarding the higher DCA-input rate in elderly persons, it might be suggested that bacterial overgrowth with anaerobes and 7α-dehydroxylation of CA in the small intestine contributes to DCA-absorption at that level in the enterohepatic bile acid circulation. It has been demonstrated, however, that such a 7α-dehydroxylation occurs only in those patients with a contaminated bowel syndrome who suffer from steatorrhoea [22]. In our subjects no signs of fat malabsorption were noted. Moreover, in view of the rather weak correlation between CA-fractional turnover rate and DCA-input rate ($n=22$, $r=+0{\cdot}53$), especially in the group of young adults ($n=11$, $r=+0{\cdot}22$), other factors seem to be involved as well as a cause of the lower DCA-input rate in group A as will be discussed below.

Gut transit time

Transit times obtained with the pellet method were often found to be shorter compared to the $^{51}CrCl_3$ method (in ten out of twenty-two subjects, mainly in those with rapid gut transits). In only one subject the intestinal transit time obtained with the $^{51}CrCl_3$ method exceeded the time measured with the pellet method. This significant difference ($P<0{\cdot}05$) is in agreement with that reported by Hinton *et al.* [10]. We preferred to use the results of the pellet method in relation to the data of bile acid metabolism, since it is assumed that pellets are travelling through the gut at the same rate as food residues which have absorbed a

great part of bile acids. Transit times of both groups were unexpectedly almost similar and therefore they do not seem to explain the differences in bile acid metabolism. The most conspicuous differences in DCA-input rate between participants of both groups were recorded in persons with rapid intestinal transit. This means that rapid intestinal transit might lower exposure of colon mucosa to bile acids in young adults.

Dietary composition

Average daily intake of vegetable protein, carbohydrates and dietary fibre was higher in the group of young adults (Table 3). In view of a lack of correlation between this and DCA-input rate, it is unlikely that the differences in DCA-input rate are directly linked to one of these dietary components. However, among other factors, dietary composition seems to be involved in the regulation of the gut transit time. This particularly applied to our young subjects. The importance of each of these factors is difficult to assess separately [5].

If differences of faecal bile acid composition in different age groups are absent as reported by Hill [23], then it may be speculated that a higher F(D) and DCA-input rate in elderly persons are to be ascribed not only to their higher CA-fractional turnover rate, but also to altered properties of the large intestinal wall, facilitating passive absorption of DCA. More extensive contact of colonic mucosa with DCA due to an increased intracolonic DCA formation might favour the absorptive process of DCA itself.

If DCA-input rate indicates exposure of the large intestine to bile acids it appears that this can be limited in young adults, but not in elderly persons by a rapid gut transit. In view of the differences in DCA input

between elderly persons and young adults, it would now be of interest to study cholic and deoxycholic acid metabolism and gut transit times in patients with increased risk for colorectal cancer.

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CHAPTER V

COLONIC ABSORPTION OF SECONDARY BILE-ACIDS IN PATIENTS WITH ADENOMATOUS POLYPS AND IN MATCHED CONTROLS

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Summary Colonic absorption of deoxycholate and the proportion of secondary bile-acids in duodenal bile were significantly higher in 11 patients with adenomatous polyps than in 11 controls matched for age, sex, and gut transit time. In the patients with adenomas the large bowel made a greater contribution to bile-acid conservation, and this was associated with a more rapid turnover of cholic acid, but not with the adenoma patients' higher consumption of cholesterol. These data accord with the hypothesis that bacterially degraded bile-salts are involved in the adenoma-carcinoma sequence of the colon.

Introduction

ADENOMATOUS polyps, particularly large ones, are regarded as precancerous lesions.[1-3] Although environmental factors, especially diet, are believed to influence the incidence of large-bowel cancer,[4,5] there is still disagreement about which substances are involved in the development of adenomas and carcinomas of the colon.[6,7]

High consumption of animal fat may be important because it increases bile-acid excretion.[8,9] Exposure of the large bowel to bacterially degraded bile-salts enhanced carcinogenesis in rats.[10] High dietary fibre intake may protect against malignancy by accelerating intestinal transit, diluting colonic

contents, and lowering pH.[11-13]

Faecal concentrations of deoxycholate (DCA), a major metabolite of cholic acid (CA), and faecal 7α-dehydroxylase activity, which is responsible for DCA formation, were higher in patients with colonic adenomas than in controls.[14-16]

We view absorption of DCA from the colon as a measure of the exposure of large-bowel mucosa to bacterial bile-acid products. We have measured DCA absorption in patients with adenomatous polyps at high risk of colon cancer and have compared these results with those in controls. Patients and controls were matched for age and gut transit time, since these factors have been shown to influence DCA absorption.

We assessed DCA absorption by measurement of the rate that DCA entered the circulating bile-acid pool. We also collected data on the diet in both groups to determine whether this was related to bile-acid metabolism.

Patients and Methods

Experimental procedure.—Eleven patients with histologically proven adenomatous polyps were investigated. In all the risk of colon cancer was high because they had severe epithelial dysplasia, large adenomas (mean diameter [±SD] 1·5±0·7 cm), or numerous adenomas.[2] Three patients had undergone cholecystectomy. None of the patients with adenomas were taking any medication or had any other illness of any note. Eleven healthy volunteers matched for age, sex, and gut transit time were studied as controls Subjects were included in the trial only if they had guaiac-negative stools; non-compromised intestinal hepatic, renal, and gallbladder function; and normal fasting serum lipids (table I). Drugs were not allowed. Previous use of laxatives, sedatives, hypnotics, antibiotics, and oestrogens was investigated. All subjects were studied as outpatients. Food-intake in the week before the study was standardised so that individual intake was known and could be maintained during the study. All foodstuffs were weighed carefully before consumption. Dietary constituents were calculated from Dutch food tables.[17]

TABLE I—DATA ON STUDY GROUPS (MEAN±SD)

—	Patients	Controls	p
No. of subjects	11	11	
Age (yr)	52·6±11·2	53·1±14·3	NS
Sex ratio (M/F)	5/6	5/6	
Δ Ideal weight (%)*	+3·6±10·8	+2·7±15·9	NS
Serum triglyceride (mmol/l)	1·16±0·44	1·37±0·55	NS
Serum cholesterol (mmol/l)	5·2±0·9	5·7±1·0	NS
Gut transit time (h)	79±39	78±37	NS

*Actual weight as percentage of ideal weight: $\frac{\text{weight (kg)}}{\text{length (cm)} - 100} \times 100$.

NS = not significant.

CA and DCA kinetics were investigated by means of a double-isotope dilution method.[18] After intravenous administration of 40 μCi (2,4-^{3}H) cholic acid and 10 μCi sodium (24-^{14}C) deoxycholate on the first day, fasting duodenal bile (2 ml) was aspirated on the next 5 consecutive days after cholecystokinin-induced gallbladder contraction. During the same period gut transit time was measured by means of radio-opaque pellets.[16]

Analyses.—We used gas/liquid chromatography to determine the concentration of CA and DCA in each bile sample after separation of both bile-acid fractions and conversion into trifluoroacetate derivatives of their methylesters.[18,19] Another volume of each bile sample was used to determine ^{3}H and ^{14}C radioactivity.[18] From semilogarithmic specific activity decay curves of ^{3}H-CA and ^{14}C-DCA we calculated pool sizes and fractional turnover rates.[20,21] From these we derived CA synthesis rates and DCA input rates. Biliary bile-acid composition (including lithocholic acid, chenodeoxycholic acid, CA, and DCA) was also measured by means of gas/liquid chromatography). Fasting serum lipids were analysed as described elsewhere.[22,23] We used Wilcoxon's rank sum test[24] and linear regression analysis[25] to compare the patients and controls.

Results

DCA and CA metabolism and biliary bile-acid composition.—

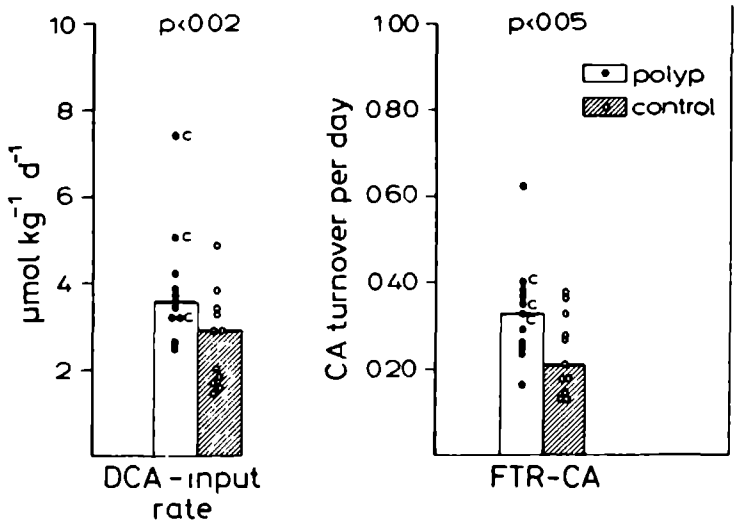

Fig. 1—Medians and individual values of DCA input rates and fractional turnover rates (FTR) of CA in patients with adenomatous polyps and matched controls.

c = previous cholecystectomy.

TABLE II—CA AND DCA METABOLISM IN PATIENTS WITH ADENOMATOUS POLYPS AND IN CONTROLS (MEDIAN AND RANGES)

	Patients	Controls	p
Cholic acid:			
Pool (μmol kg^{-1})	35·4 (7·9–63·4)	42·5 (14·7–57·8)	NS
Synthesis rate (μmol kg^{-1}d^{-1})	8·2 (5·0–25·4)	7·7 (5·4–15·4)	NS
Fractional turnover rate (d^{-1})	0·33 (0·16–0·63)	0·21 (0·13–0·38)	<0·05
Deoxycholic acid:			
Pool (μmol kg^{-1})	20·9 (10·1–25·9)	13·4 (6·1–37·7)	NS
Input rate (μmol kg^{-1}d^{-1})	3·6 (2·5–7·5)	2·9 (1·5–4·9)	<0·02
Fractional turnover rate (per day)	0·17 (0·12–0·29)	0·22 (0·09–0·30)	NS
Total bile acid pool (μmol kg^{-1})	82·2 (49·6–134·1)	94·1 (50·6–147·0)	NS

NS = not significant.

DCA input rates were significantly higher in adenoma patients than in controls (medians 3·6 and 2·9 μmol $kg^{-1}d^{-1}$ respectively, $p<0·02$) as were the fractional turnover rates of CA pools (medians 0·33 and 0·21 per day, $p<0·05$) (fig. 1). However, a tendency to larger DCA pools and smaller CA pools in the adenoma patients did not achieve statistical significance (table II). The proportion of secondary bile-acids (DCA plus lithocholic acid) in the bile of adenoma patients seemed to be increased at the expense of the primary bile-acids, particularly CA, when compared to that of the controls (medians 31·0% and 24·6%, respectively, $p<0·05$ for secondary bile-acids, and 31·7% and 38·5%, respectively, $p<0·05$ for CA [fig. 2]). DCA input rate was more closely associated with CA metabolism (CA synthesis rate, CA fractional turnover rate, and CA pool) in adenoma patients than in controls subjects (table III).

Dietary composition.—Cholesterol intake was higher in adenoma patients than in controls (table IV). However, cholesterol intake was not related to CA or DCA metabolism.

TABLE III—CORRELATIONS BETWEEN CA METABOLISM AND COLONIC DCA ABSORPTION

	Patients		Controls	
	r	p	*r*	p
CA pool *vs* DCA input rate	0·72	<0·05	−0·17	NS
CA synthesis rate *vs* DCA input rate	0·96	<0·001	0·44	NS
FTR CA *vs* DCA input rate	0·68	<0·05	0·43	NS

FTR = fractional turnover rate.

TABLE IV—DIETARY CONSTITUENTS IN STUDY GROUPS (G/DAY)
(MEAN±SD AND RANGE)

—	Patients	Controls
Total protein:	80±19 (48–106)	71±15 (51–102)
Animal	59±16 (33–81)	51±12 (31–70
Vegetable	21±7 (8–31)	20±5 (10–32)
Total fat:	91±31 (33–137)	78±13 (47–92)
Animal	72±31 (20–127)	63±15 (36–80)
Vegetable	19±16 (7–52)	14±11 (3–42)
Cholesterol (mg/day)	315±171* (112–577)	196±35* (138–233)
Saturated and mono-unsaturated fat	77±25 (39–119)	65±12 (38–78)
Polyunsaturated fat	12±6 (5–22)	11±5 (3–18)
Carbohydrates	233±64 (112–347)	218±53 (129–341)
Dietary fibre	25±7 (10–33)	26±8 (12–39)

*$p<0{\cdot}04$. None of the other comparisons between patients and controls was statistically significant

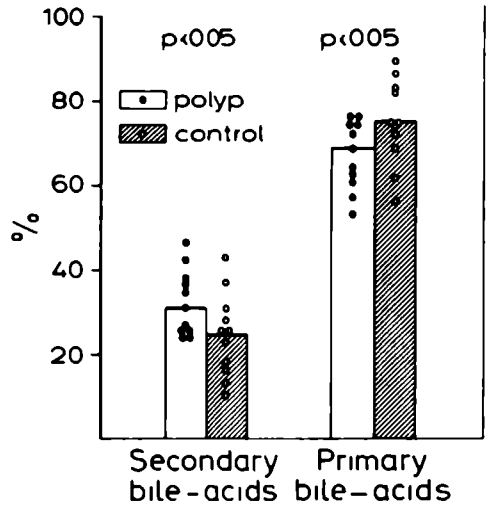

Fig. 2—Medians and individual values of proportions (% on a molar basis) of secondary bile-acids (lithocholic and deoxycholic acid) and primary bile-acids (chenodeoxycholic acid and cholic acid) in patients with adenomatous polyps and in controls.

Discussion

The finding of enhanced DCA absorption in the large bowel of patients with adenomatous polyps (fig. 1) accords with the suggested role of bile-acid conversion products in colonic carcinogenesis.

Patients and controls were matched for gut transit and age, both factors which could have influenced DCA absorption. The increased proportion of secondary bile-acids in the bile of adenoma patients accords with enhanced DCA absorption (fig. 2); it also demonstrates the greater contribution made by the colon to bile-acid conservation in these patients. However, both our data of colonic DCA absorption and the results of studies by other workers of faecal bile-acid in adenoma patients and controls showed a considerable overlap.[14,15] In view of the high prevalence of asymptomatic colonic adenomas in older patients[2] we cannot be certain, without colonoscopic or radiological investigations, that our apparently healthy controls were free of colonic adenomas. We believe that this might explain some of the overlap between results for patients and controls.

Enhanced DCA absorption in the large bowel of adenoma patients seemed to be associated with an increase in the fractional turnover rate of the CA pools (table IV). A similar combination of increased CA turnover rate and increased incorporation of DCA from the colon into the DCA pool has been described in patients after cholecystectomy.[26,27] Case-control studies have also demonstrated an increased risk of carcinoma in the right colon in women after cholecystectomy.[28,29] Three of the adenoma patients in our study had undergone cholecystectomy (fig. 1), but the difference in DCA absorption between patients and controls remained when these 3 patients were excluded. Thus it seems that increased DCA absorption in patients after cholecystectomy and in those with adenomatous polyps may indicate an increased risk of colon cancer.

Higher cholesterol consumption in adenoma patients was not associated with a higher consumption of animal fat or a lower intake of dietary fibre. This seems to accord with the close correlation between colon cancer mortality and cholesterol intake revealed by analysis of international food consumption data.[30] There is experimental evidence of a co-carcinogenic effect of high cholesterol consumption on the induction of colon cancer in rats,[31] but the underlying mechanism remains obscure. Restriction of protein and fat intake reduced CA turnover rate, and this was attributed to decreased gallbladder stimulation and a lower circulation frequency of the bile-acid pool.[32] Our study does not suggest that cholesterol intake, which was higher in adenoma patients than in controls, had a similar influence on CA metabolism. The similarity of total bile-acid pool sizes in both groups (table II) does not suggest important differences in their enterohepatic circulation rates.[33] Therefore a less efficient ileal absorption of CA in adenoma patients must be responsible for their more rapid CA fractional turnover rate.

In our patients with adenomatous polyps an increase in DCA absorption was closely associated with an increased turnover rate of their CA pool. Further work is needed to identify which factors are responsible for the apparently less efficient CA conservation in these patients.

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CHAPTER VI

INTRACOLONIC ENVIRONMENT AND THE PRESENCE OF COLONIC ADENOMAS IN MAN

Abstract

A promoting effect of large-bowel contents on colonic carcinogenesis as seen in the animal model is still incompletely explored in man. In our study, we investigated simultaneously deoxycholate absorption (as marker of colonic mucosal exposure to tumour-promoting bile-salt metabolites), mouth-anus transit time and the ratio of anaerobic to aerobic bacteria in the stools of ten patients with colonic adenomas and ten age-matched control subjects. We found that anaerobic/aerobic ratios and colonic deoxycholate absorption were higher in patients with colonic adenomas ($p < 0.002$ and $p < 0.001$), and that these parameters were clearly interrelated, which also applied to intestinal transit times and the anaerobic/aerobic ratios. These data are consistent with a promoting effect of the intracolonic environment on the development of adenomas in man. Long-term induction of a more aerobic colon flora and shortening of intestinal transit time may diminish bile-salt induced tumour-promotion in adenoma patients.

Introduction

High faecal bile-acid concentrations in the presence of large numbers of some species of anaerobic bacteria, able to dehydrogenate the bile-acid nucleus, have been reported in patients with cancer of the colon[1,2]. This has led to renewed interest in the theory that formation of (co)carcinogenic bile-acid metabolites in the large bowel and subsequent mucosal exposure to these products may be involved in colonic carcinogenesis in man[3]. Later studies could not confirm that patient groups with colonic carcinoma or with colonic adenoma (where the risk of colon cancer is high) could be identified by counts of bile-acid degrading bacteria[4,5]. However, both 7α-dehydroxylation, the initial step in bile-acid nucleus conversion, and the ratio of anaerobic to aerobic (i.e. facultative anaerobic) bacteria proved to be increased in their stools[4,6] reflecting better growth conditions and higher enzymatic activity (in vitro) of their anaerobic faecal flora.

The view that colonic stasis evidenced by a slow gut transit may have an additional influence is still controversial[7,8]. Other data supporting the bile-acid hypothesis have been confined almost exclusively to animal and in vitro experiments[9,10].

In our studies of colonic carcinogenesis in man, we employed endogenous deoxycholate (DCA) absorption as a marker of mucosal exposure to secondary bile-acids. DCA-absorption can be assessed from the daily DCA-input into the circulating bile-acid pool using isotopic bile-salt studies in bile [11,12]. An enhanced colonic DCA-absorption could certainly be demonstrated in adenoma patients[13]. Another study objective was to investigate possible relationships of colonic DCA-absorption with quantitative data of the anaerobic and aerobic faecal flora as indicators of the colonic microenvironment[14].

This report describes these additional findings in the high risk adenoma patients compared with control subjects matched for age and (as closely as possible) also for intestinal transit time - because of their influence on DCA absorption shown previously[15].

Methods

Subjects

Ten patients with histologically proved colonic adenomas participated in the study. In all the risk of colon cancer was high on account of severe epithelial dysplasia (5 patients) and/or size (mean diameter ± S.D. : 1.5 ± 0.7 cm) and number of their adenomas (6 patients with two or more recurrent adenomas)[16], but none suffered from adenomatous polyposis or had any other illness. No laxatives, antibiotics, sedatives, hypnotics or oestrogens were used. Ten healthy volunteers without medication, matched for age and with comparable gut transit times served as control subjects. Eligibility required guiac-negative stools, a non-compromised intestinal, hepatic, renal and gallbladder function and normal fasting serum lipids. Basal data are given in Table 1.

TABLE 1 BASAL DATA ON INVESTIGATED SUBJECTS (MEAN ± S.D.)

	Patients	Controls	P
Number of subjects	10	10	
Age (year)	50 ± 9	50 ± 9	
Sex ratio (♂/♀)	6 / 4	5 / 5	
Relative weight (%)*	103 ± 11	105 ± 18	N.S.
Serum triglyceride (mmol/l)	1.43 ± 0.54	1.19 ± 0.76	N.S.
Serum cholesterol (mmol/l)	5.8 ± 1.0	4.7 ± 1.1	< 0.05
Gut transit time (hour)	72 ± 31	67 ± 40	N.S.

N.S. : not significant

*as compared to ideal weight : $\frac{\text{actual weight (kg)}}{\text{length (cm)} - 100} \times 100$.

Study design

All subjects were studied as outpatients. Food intake, starting one week prior to the isotopic bile-salt study, was individually and carefully standardized[15]. Each participant passed in this period one stool, using the laboratory lavatory, which allowed immediate microbiological processing. DCA-metabolism was subsequently investigated using a previously reported isotope dilution method[11,12]. Following intravenous administration of 10μCi sodium [24-^{14}C] deoxycholate, fasting duodenal bile (less than 2 ml) was aspirated on the next five mornings after cholecystokinin-induced gall-bladder contractions.

Mouth-anus transit time was measured during this period with radio-opaque pellets[15,17].

Analyses

Bile

Pool sizes and fractional turnover rates of DCA, allowing calculation of DCA-inpute rate, were derived from the semilogarithmic specific activity decay curves in bile[18].

The required measurements of DCA in each bile sample (0.5 ml) were performed by gas-liquid chromatography after purification by thin layer adsorption chromatography and conversion into trifluoroacetate derivatives of its meythyl esters[19,20]; ^{14}C-radioactivity was determined by scintillation counting[12].

Serum lipids

Previously reported methods of examining fasting serum lipids were employed[21,22].

Faecal microbiology

A weighted stool sample of approximately one gram was introduced without delay into an anaerobic glove box[23]. After rapid preparation of a series of faeces dilutions in saline, amounts of 100 µl were pipetted from the appropriate homogenized suspensions ($10^{-5},^{-6},^{-7}$ and $10^{-8},^{-9},^{-10}$ for aerobic and anaerobic cultures respectively)and spread onto an agar medium, enriched with sheep blood (1 to 2 days), the other one anaerobically (1 week)[25]. The total counts of colony-forming units are given as their logarithms[26].

Direct microscopic counts were obtained according to Holdeman and Moore [27] and compared with the total of anaerobic colony-forming units to define the recovery of vital anaerobes. Culture yields of less than 15% were not reproducible and were not accepted for evaluation (Table 2).

Faecal pH was measured in another part of the stool after saline dilution (v/v : 1/1) using a pH electrode (Radiometer Copenhagen Pm22r).

Statistics

Differences between both groups were analysed with the Wilcoxon Rank Sum test, significance of associations was derived from Spearman correlation coefficients[28].

Results

Input rates of DCA into the bile-acid pool were higher in adenoma patients than in control subjects (medians 3.6 and 1.9 µmol $Kg^{-1}d^{-1}$, $p < 0.001$, Table 2 and Fig 1), whereas total anaerobic and aerobic counts showed no significant distinction (Table 2).

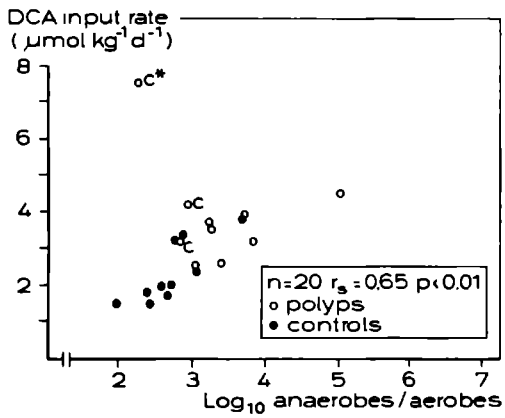

Figure 1 : Scatter diafram of anaerobic/aerobic ratio and deoxycholate absorption in patients with adenomatous polyps (o) and control subjects (•).
C : previous cholecystectomy
* : see text (discussion)
DCA : Deoxycholate

Table 2 DATA ON COLONIC DEOXYCHOLATE ABSORPTION, FAECAL MICROBIOLOGY, FAECAL pH AND FAECAL WATER CONTENT IN PATIENTS WITH ADENOMATOUS POLYPS AND CONTROL SUBJECTS (MEDIANS AND RANGES).

	Patients (n=10)	Controls (n=10)	P
Deoxycholate absorption (µmol $Kg^{-1}d^{-1}$)	3.6 (2.5 - 7.5)	1.9 (1.5 - 3.8)	<0.001
Total anaerobic bacteria (g^{-1}dry weight)*	11.31 (10.49-11.49)	11.07 /10.23-11.87)	N.S.
Total aerobic bacteria (g^{-1}dry weight)*	8.16 (5.62-8.64)	8.20 (7.56-9.92)	N.S.
Anaerobic/aerobic ratio*	3.22 (2.23-5.00)	2.66 (1.95-3.68)	<0.002
Recovery of vital anaerobes (%)	30 (15 - 81)	38 (15 - 97)	N.S.
Faecal pH	6.9 (6.2 - 8.0)	7.1 (6.7 - 7.5)	N.S.
Faecal water	72 (66 - 78)	76 (66 - 83)	N.S.

N.S. : not significant
* : Data transformed into Log_{10}.

The clear relationship between the anaerobic/aerobic ratio and DCA-absorption (r_s= 0.65, p < 0.01, Fig.1) was in contrast to a lack of correlation of both anaerobic and total aerobic counts with colonic DCA absorption (both r_s < 0.37, N.S.). No difference in faecal pH was observed (Table 2); however, slower intestinal transit times were associated with higher anaerobic/aerobic ratios (Fig.2).

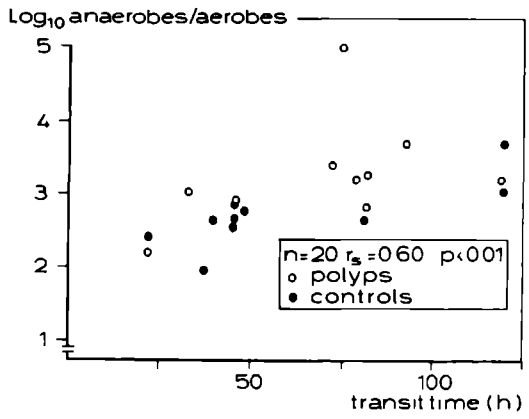

Figure 2 :

Scatter diagram of gut transit time and anaerobic /aerobic ratio in patients with adenomatous polyps (o) and control subjects (•).

Discussion

This study shows an enhanced DCA-absorption and a greater predominance of the anaerobic flora (as judged from the anaerobic/aerobic ratio) in patients with colon adenomas. In addition, it demonstrates that these parameters are interrelated, which applied to patients as well as to control subjects (Fig 1, Table 2). This favours a link between a colonic well suited to the growth of anaerobic bacteria and exposure of colonic mucosa to tumour-promoting bile-acid metabolites. The results of this study are also consistent with epidemiological reports of relatively low ratios of anaerobic to aerobic faecal bacteria in populations from Africa and Asia with a low risk of colon cancer[29]. The marked predominance of faecal anaerobes in adenoma patients is in agreement with other studies[4,8].

Both low and high anaerobic/aerobic ratios have been described in faecal cultures of colon cancer patients[8,30]. These apparently conflicting results can be reconciled by assuming that large-bowel tumour may accelerate gut transit in some cases, which appears to be accompanied by a lower anaerobic/aerobic ratio of the gut flora according to our data. This may apply to those colon cancer patients with a history of more frequent bowel motions. Three of the adenoma patients had had a previous cholecystectomy, another possible risk factor of colon cancer justifying their inclusion into the patient group.

Exclusion of the data from these subjects did not essentially affect the statistical analysis of the results reported.

The rapid intestinal transit in one of our cholecystectomized adenoma patients (* Fig.1)seems to be secondary to an increased turnover of her bile-acid pool[13], a possible effect of cholecystectomy[31]. The ensuing increase in DCA formation enhances colonic water secretion[32] and intestinal motility[33] and also suppresses growth of anaerobic bacteria[34], which may account for the rapid transit and low anaerobic/aerobic ratio in this patient.

Our finding that a rapid mouth-anus intestinal transit was associated with a more aerobic flora in all subjects (Fig.2) could be ascribed to a lack of extensive intracolonic stasis. Experimental data on anaerobic colonization of self-filling, but not of self-emptying blind loops leave no doubt about such an effect of intestinal stasis.

The impact of slow intestinal transit on colonic carcinogenesis as emphasized by Burkitt[6] may be attributed to a more anaerobic intracolonic environment, which appears to enhance mucosal exposure to secondary bile acids (Fig.1). Although the mean intestinal transit of our adenoma patients was slower than in our control subjects (Table 1) the difference was small and not statistically significant. In our opinion, it may be a contributory but not the sole underlying mechanism of the higher DCA-absorption in our adenoma patients. This is in accordance with our earlier observation that DCA-absorption and gut transit time are only clearly correlated in young adults[15].

Rapid formation of DCA in the colon is also dependent on an alkaline pH and may facilitate DCA absorption from the colon. Since it is unlikely that faecal pH will always reflect the pH in the colon, we were not surprised that no correlation could be detected between DCA absorption from the colon and the pH in a fresh stool sample of our subjects (Table 2).

In conclusion, this study reveals a relationship between DCA absorption and anaerobic growth conditions in the large bowel and gut transit. Mucosal exposure to bile-acid metabolites appeared to be higher in patients with adenomas and was associated with their more anaerobic gut flora as compared to control subjects. These data suggest that induction of a more aerobic bacterial flora may lead to a less tumour-promoting environment in the large bowel, a possibility which should be further explored.

Previous observations on the influence of dietary composition on faecal flora have shown that this may require rather long term studies[35,36].

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CHAPTER VII

ABSORPTION OF ENDOGENOUS DEOXYCHOLATE IN RELATION TO BILIARY BILE-ACID COMPOSITION AND CHOLESTEROL SATURATION IN MAN.

ABSTRACT

The hypothesis that absorption of endogenous deoxycholate from the colon may enhance cholesterol saturation in bile and cholesterol gallstone disease by selective inhibition of chenodeoxycholate synthesis has been tested. We investigated biliary lipids and bile-acid composition in a group of healthy persons with considerable differences in colonic deoxycholate absorption (elderly persons and young adults with, respectively high, and low deoxycholate absorption). Cholic acid metabolism was measured simultaneously with deoxycholate absorption using a double isotope dilution technique.

Deoxycholate absorption, in contrast to the degree of overweight, was not related with cholesterol saturation in bile or with the proportion of cholesterol in biliary lipids. Extensive deoxycholate absorption in elderly persons was reciprocally associated with the proportion of chenodeoxycholate in biliary bile-acids. With regard to cholic acid metabolism we noted that in spite of the more rapid fractional turnover rates of cholic acid in elderly persons their cholic acid synthesis was not higher than that of young adults. This may suggest partial suppression of cholic acid synthesis. These data do not confirm the fact that deoxycholate absorption induces cholesterol supersaturation in bile to any considerable extent ; they do, however, seem to be consistent with the influence of deoxycholate absorption on the metabolism of both primary bile-acids rather than with the selective suppression of the synthesis of chenodeoxycholate only.

INTRODUCTION

It has been noted that the bile of patients with gallstones contains a rather high proportion of deoxycholate (DCA)[1]. This has led to speculation that increased colonic absorption of this bacterial metabolite of cholic acid (CA) might predispose to the formation of cholesterol stones[2].

Supporting evidence has been produced by the oral administration of low doses (100 - 150 mg daily) of DCA to healthy subjects, which raised cholesterol saturation in bile, whereas biliary bile-acid composition showed an increase of DCA at the expense of chenodeoxycholate (CDCA)[3,4]. Opposite effects have been reported after ingestion of metronidazole[5] or lactulose[6].

Isotopic bile-salt studies in four subjects on low doses (100-150 mg daily) of DCA revealed, in contrast to the suppression of primary bile-salt synthesis (of CA and CDCA)during high oral doses (750 mg daily) of DCA[7], a consistently diminished synthesis of CDCA, but not of CA[4].

It has been suggested, that also in the natural situation an increase in colonic absorption of endogenous DCA may cause cholesterol supersaturation in bile by selective inhibition of hepatic CDCA-synthesis[4]. However, exploration of the relation between various levels of colonic DCA-absorption and biliary lipid composition offers a more direct and more physiological approach of the natural situation than investigation of the effect of oral ingestion of low doses of DCA.

This report describes, therefore, data on cholesterol saturation and bile-acid composition in bile of healthy subjects with a wide range of colonic DCA-absorption (young adults and elderly persons with respectively low and high DCA absorption as shown previously[8]). Colonic DCA-absorption can be assessed from the daily DCA-input rate into the circulating bile-acid pool by isotopic bile-salt studies. To find out whether inhibition of CDCA synthesis by colonic DCA absorption could be really selective, as was suggested, we reinterpreted the (simultaneously obtained) data on CA metabolism which were already reported earlier in a different context[8].

In addition, the influence of DCA absorption has been compared with that of relative bodyweight (as measure for overweight , a common promoting factor of cholesterol gallstone disease[9]).

SUBJECTS AND METHODS

Subjects

Two groups of 11 healthy volunteers age participated in the study after informed written consent. Eligibility required a non-compromized intestinal, hepatic,renal and gallbladder function (the latter was ascertained by a dark brown colour of duodenal bile after gallhladder stimulation).

When any suspicion of cholelithiasis existed (in 2 elderly subjects) oral cholecystography was performed. Persons with any medication, hyperlipidaemia, gross obesity (more than 150% of ideal weight) or previous major intestinal surgery were not included[8]. Basal data are given in table I.

TABLE I BASAL DATA OF SUBJECTS (MEAN ± S.D.)

	Young adults	Elderly persons	P
Number of subjects	11	11	
Age	21.8 ± 1.8	67.3 ± 4.5	
Sex (male/female)	5 / 6	6 / 5	
Weight (Kg)	67.6 ± 5.1	69.1 ± 12.3	N.S.
*Relative weight (%)	87 ± 8	111 ± 17	< 0.001
Serum triglyceride (mmol/l)	1.03 ± 0.41	1.56 ± 0.73	N.S.
Serum cholesterol (mmol/l)	4.6 ± 1.0	5.4 ± 1.0	< 0.05

*as compared to actual weight :

$$\frac{\text{actual weight (kg)}}{\text{length (cm)} - 100} \times 100\% \ ;$$

N.S. : not significant.

Experimental design

Subjects were studied as out-patients. Daily food intake was, starting one week prior to the study, carefully standardized according to individual habits[8].

Bile-acid metabolism was investigated using a previously reported double isotope dilution method[8]. Following intravenous administration of forty μCi [2,4 - ^{3}H] cholic acid (specific activity : 16 Ci.mmol^{-1}) and ten μCi sodium [24 - ^{14}C] deoxycholate (specific activity : 0.052 Ci. mmol^{-1}) (both more than 97% pure according to thin layer chromatography), duodenal bile (less than 2 ml) was aspirated after cholecystokinin induced gallbladder contraction on the next five mornings. Immediately after collection one half ml was dissolved in 4.5 ml isopropanol for determination of biliary

lipids (in ten subjects of each group) and stored like the remainder of the bile sample at -20°C.

Analyses

Pool sizes and fractional turnover rates of DCA and CA allowing calculation of DCA-input rate and CA-synthesis rate were derived from their semilogarithmic specific activity decay curves in bile(linear correlation coefficients 0.99 ± 0.01)[10,11]. The required measurements of DCA and CA and total bile-acid composition in each bile sample (0.5 ml) were performed by gas-liquid chromatography after separation of both bile-acids by thin layer adsorption chromatography and conversion into trifluoroacetate derivatives of their methyl esters[12,13]; radioactivity was determined by fluid scintillation counting. These methods have been described in detail[8,14] as were those used for examination of fasting serum lipids[15,16].

Two most concentrated isopropanolic bile solutions were analyzed in each subject[14]. Phospholipids were measured in duplicate in 0.1 ml as lipid soluble phosphorus[17] and cholesterol and total bile acids in two other volumes of 0.2 ml using an enzymatic colorimetric assay (Boehringer Mannheim GmbH, Germany[18]) and a 3α-steroid -dehydrogenase method[19]. Cholesterol saturation indices in bile were derived from polynomial equations developed by Thomas and Hofmann[20] to describe the cholesterol solubility line as proposed by Hegardt and Dam[21] and Holzbach et al[22].

Statistics

Differences between both groups were analysed with the Wilcoxon rank sum test with confidence level at 0.05[23] using a Texas instruments calculator PC-100B. Linear regressions have been calculated by the method of least squares; their significance has been obtained from Pearson's correlation coefficient r, which accorded nicely with the non-parametric method of Spearman in our data[24].

RESULTS

Bile of elderly persons, known to have higher DCA-input rates than young adults (medians 3.1 and 1.9 μmol/l Kg^{-1} d^{-1}, $p < 0.005$), also showed a bile-acid composition with more DCA (medians 24.6% and 10.6%, $p < 0.001$) (Fig. 1 and Table II).

Table II DATA ON CHOLATE AND DEOXYCHOLATE METABOLISM (MEDIANS AND RANGE)

	Young adults n= 11	Elderly persons n= 11
FTR CA pool (d^{-1})	0.22 (0.08 - 0.43)	0.31 (0.18 - 0.44)*
FTR DCA pool (d^{-1})	0.23 (0.09 - 0.51)	0.24 (0.11 - 0.35)
CA pool size (μmol.Kg^{-1})	37.3 (19.3 - 91.7)	31.0 (16.9 - 53.9)
DCA pool size (μmol.Kg^{-1})	7.6 (0.5 - 17.9)	13.4 (5.9 - 36.5)**
CA synthesis rate (μmol.Kg^{-1}.d^{-1})	7.6 (5.3 - 17.9)	9.6 (5.4 - 19.8)
DCA input rate (μmol.Kg^{-1}.d^{-1})	1.9 (0.2 - 2.9)	3.1 (1.5 - 5.8)**

FTR : fractional turnover rate
CA : cholic acid
DCA : deoxycholic acid
* : $p < 0.025$
** : $p < 0.005$

Although a tendency in the elderly group to lower proportions of CDCA in the biliary bile-acids was not significant (Fig.1), there was actually a difference in cholesterol saturation : four out of ten young adults had supersaturated bile, compared with eight out of ten elderly persons ($p < 0.05$, Fig. 1).

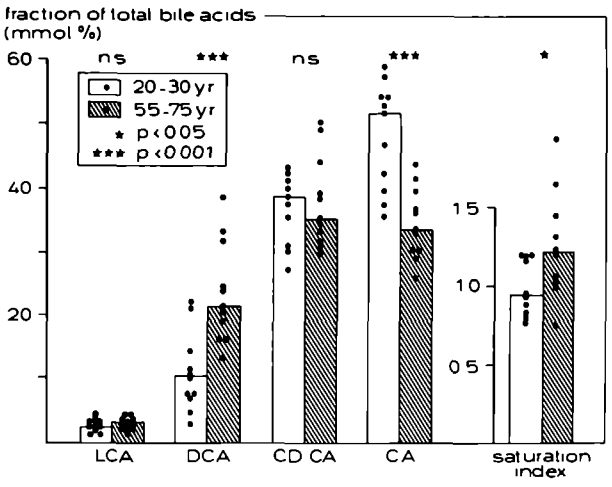

Figure 1: Bile-acid composition and cholesterol saturation index in bile of young adults and elderly persons. Medians are indicated by column height, individual values by symbols.

LCA : lithocholate
DCA : deoxycholate
CDCA : chenodeoxycholate
CA : cholic acid

A negative correlation of DCA-input rates and the CDCA bile-acid fractions was also found, but proved to be confined to elderly subjects only ($r = -0.65$, $p < 0.05$, fig. 2).

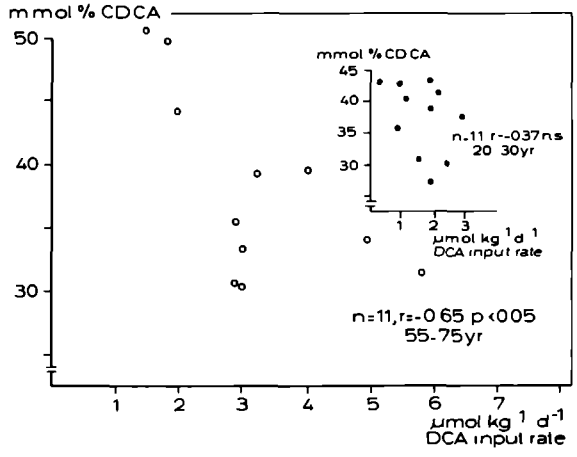

Figure 2 : Scatter diagram of deoxycholate input rate and the proportion of chenodeoxycholate in the biliary bile-acid composition in elderly persons and in young adults (inset).
DCA : deoxycholate
CDCA : chenodeoxycholate
n.s. : not significant.

However, no relationship at all was discovered between DCA-input rate and cholesterol saturation indices or one of the biliary lipid fractions (Table III), despite the significant differences between both groups in these respects (Table IV).

TABLE III CORRELATIONS OF COLONIC DCA ABSORPTION AND RELATIVE BODY-WEIGHT TO CHOLESTEROL SATURATION INDEX AND BILIARY LIPID FRACTIONS

COLONIC DCA ABSORPTION - VERSUS -	Young adults n = 10		Elderly persons n = 10		All subjects n = 20	
	r	p	r	p	r	p
Cholesterol saturation index	-0.12	N.S.	-0.20	N.S.	+0.13	N.S.
Cholesterol (mmol%)	-0.10	N.S.	-0.35	N.S.	+0.16	N.S.
Phospholipids (mmol%)	+0.14	N.S.	-0.58	N.S.	+0.25	N.S.
Bile-acids (mmol%)	-0.24	N.S.	+0.48	N.S.	-0.21	N.S.
Relative body-weight (%)	-0.12	N.S.	-0.20	N.S.	+0.13	N.S.
RELATIVE BODY-WEIGHT - VERSUS -						
Cholesterol saturation index	+0.16	N.S.	+0.79	<0.01	+0.73	<0.001
Cholesterol (mmol%)	+0.22	N.S.	+0.80	<0.01	+0.83	<0.001
Phospholipids mmol%)	+0.01	N.S.	+0.76	<0.01	+0.83	<0.001
Bile-acids (mmol%)	-0.75	<0.05	-0.81	<0.01	-0.90	<0.001

DCA : deoxycholate
Relative body-weight : see Table I.

TABLE IV CHOLESTEROL SATURATION INDEX AND TOTAL BILIARY LIPIDS AND LIPID FRACTIONS (MEDIANS AND RANGES)

	Young adults n=10	Elderly persons n = 10	P
Cholesterol-saturation index	0.95 (0.79 - 1.19)	1.22 (0.76 - 1.93)	<0.05
Cholesterol fraction (mmol%)	5.4 (3.9 - 7.0)	8.5 (5.2 - 16.2)	<0.005
Phospholipid fraction (mmol%)	18.8 (13.8 - 18.6)	23.2 (16.2 - 30.6)	<0.001
Bile-acid fraction (mmol%)	77.1 (72.3 - 80.2)	68.1 (53.2 - 78.2)	<0.001
Total biliary lipids (mmol/l)	118.8 (47.2 - 169.3)	53.3 (20.2 - 124.1)	N.S.

CA-metabolism showed a more rapid fractional turnover rate of CA pools in elderly persons (medians 0.31 $.d^{-1}$ versus 0.22 $.d^{-1}$) accompanied by some increase of CA-synthesis rate and some decrease of CA-pool size, both without statistical significance (Table III).

In marked contrast to the lack of any association between relative body weight and DCA-input rate was the close relationship of relative body weight with cholesterol saturation indices and with all three biliary lipid fractions (Table III). A relationship was also noted between DCA-input rate and CA-fractional turnover rate (n=22, $r = +0.53$, $p < 0.01$).

DISCUSSION

Our data cast some doubt on the hypothesis (extrapolated from the effects caused by the feeding of DCA in low doses and from studies with lactulose and metronidazole) that also in the natural situation an increase in colonic absorption may elicit an important rise in cholesterol saturation in bile, thereby predisposing to the formation of gallstones[4,5,6].

The inverse relation of DCA-input rate and the proportion of CDCA in biliary bile-acids may be consistent with an inhibition of CDCA synthesis by a high colonic DCA absorption, which has been proposed as underlying mechanism. However, it remains doubtful whether this inhibition is really selective as has been suggested, since it appeared that CA-metabolism was also affected. A more rapid fractional turnover rate of CA-pools in elderly persons would be expected to enhance CA-synthesis accordingly by a decrease in feedback regulation to maintain a constant pool size[25]. This could not be demonstrated using our data. Therefore, in our opinion, a modest inhibition of CA-synthesis may be possible. The question remains: why are our data at variance with the hypothesis under review ? This hypothesis has been based on the assumption that the influence of colonic DCA absorption on biliary lipid composition in the natural situation can be compared with the effect of low oral doses of DCA. However, this view has not been proved and, in our opinion, is probably incorrect.

If we compare relative (over)weight and colonic DCA absorption, it appears that neither are interdepedent. The changes of cholesterol metabolism induced by obesity seem far more important than colonic DCA absorption for the development of supersaturated bile. Age-dependent increases of cholesterol saturation in bile have been described for the Japanese and for Chilean and Swedish women[26,27,28]. Although our data seem to confirm this for the Dutch, it cannot be excluded, from either the quoted studies or from ours, whether a modest degree of overweight could not also be responsible for this increase in cholesterol saturation with advancing age. The coexisting relationships of the three biliary lipid fractions and relative overweight, a biliary lipid pattern which has been described in patients with obesity[9], is in favour of this view.

In conclusion, endogenous DCA absorption from the colon does not seem to determine biliary lipid composition in bile in the natural situation, at least not to the same extent as relative body-weight; indirect evidence shows that a rise in colonic DCA absorption is associated with a change in the metabolism of both primary bile-acids and not with a suppression of CDCA synthesis only.

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CHAPTER VIII

8.1 Summary and conclusions

CHAPTER I Introduction

Epidemiological data suggest that environmental influences are implicated in carcinogenesis of the large bowel in man and that feeding habits are important in this context. Since the search for complete carcinogens such as radiation, air and water pollutants, food additives or viral infections has not yielded many clues, it would seem that the relation of cancer and diet is more complex. The available information is consistent with a tumour-promoting (rather than with a tumour-initiating) role of nutrition mediated by endogenous co-carcinogenic compounds such as bacterially degraded bile-salts.

The main purpose of our study was to examine deoxycholate absorption from the colon as a potential marker of exposure of colonic mucosa to degradation products of bile-salts. Therefore, kinetic studies in bile were undertaken on the metabolism of deoxycholate, the main secondary bile-acid in human bile, and its precursor, cholic acid.

Age-dependent differences in healthy subjects (young adults and elderly persons)are described in chapter IV with regard to deoxycholate and cholate metabolism against the background of dietary composition and intestinal transit times.

A comparison of patients with colonic adenomas, where the risk of colon cancer is high, and control subjects with respect to deoxycholate absorption is made in chapter V. Differences in food consumption are also evaluated.

The relation of colonic micro-environment (judged from anaerobic and aerobic faecal cultures), intestinal transit time and deoxycholate absorption is demonstrated both in patients with adenomas and in control subjects in chapter VI.

The influence of deoxycholate absorption from the colon on biliary lipid composition (which may be recognized as an important predisposing element in cholesterol gallstone formation) is analysed from the data of healthy control subjects in chapter VII.

CHAPTER II Epidemiology and aetiology of large bowel carcinoma.

A review of the literature on recent insights in colonic carcinogenesis is presented in order to demonstrate the possible relevance of bacterial bile-salt metabolites as tumour-promoting agents in the human colon and their relation to dietary habits. It seems unlikely that bacterial transformation of large bowel contents is not relevant to the carcinogenic process. Both animal models of colonic carcinogenesis and studies from various countries on faecal sterols and faecal bacteriology in man support the role of bile-salt metabolites as endogenous tumour-promotors in the colon. The bile-salt hypothesis provides further a link between the consumption of a high fat low fibre diet in Western countries, and their high incidence of large bowel cancer.

It is unknown if tumour-promotion by bile-salt metabolites in the colon can be diminished by changing the feeding habits towards a low-fat and fibre-enriched diet. The final evidence may be provided by prospective diet-intervention studies in high risk patients with colonic adenomas.

CHAPTER III Quantification of bile-salt metabolism and intestinal transit time.

Bile-salts have a wide array of physiological functions and effects. They have a very efficient enterohepatic circulation. The primary bile-acids, cholic acid and chenodeoxycholic acid, are synthesized in the liver from cholesterol; deoxycholate, the major bacterial bile-salt metabolite in human bile is formed in the colon under influence of anaerobic bacteria.

The metabolism of these bile-salts can be studied by an isotope dilution method in bile as described by Lindstedt. This offers the possibility of measuring absorption of deoxycholate from the colon as the daily input of deoxycholate into the circulating bile-acid pool.

We used a dual isotope dilution technique in bile for simultaneous determination of cholic acid and deoxycholate metabolism. The application of [2,4 - ^{3}H] cholic acid and [24 - ^{14}C] deoxycholate as bile-acid labels gave reproducible results.

To determine mouth-anus intestinal transit time in the study, methods were employed which involve administration of non-absorbable markers in

one dose. One main advantage of these "one dose methods" in short-term studies in outpatients is that they can be easily supervised.

Solid and liquid markers do not produce the same results in the "one dose" method; liquid markers have a slower excretion rate compared to solid markers, especially in the case of a rapid intestinal transit. Radiolabelled liquid markers like $^{51}CrCl_3$ offer the possibility, if a whole-body-counter is available, to measure marker retention in the subject instead of faecal marker excretion. A whole-body-counter is therefore a useful tool to confirm completeness of stool collections.

The use of solid markers may be selected in studies of secondary bile-acid metabolism since bile-salts are tightly adsorbed to particulate matter of bowel contents.

CHAPTER IV Age-dependent differences in human bile-acid metabolism and 7α-dehydroxylation.

It has been suggested that transformation of secondary bile-acids into (co)-carcinogenic compounds play a role in the development of cancer of the large bowel. Because of age-dependent differences in the occurrence of this disease, we undertook a study of cholic and deoxycholic acid metabolism of eleven young adults (20 to 30 years) and eleven elderly persons (55 to 75 years) with a double isotope dilution method. Daily food intake was standardized individually and gut transit time measured with radio-opaque pellets and $^{51}CrCl_3$.

Results of our study show a higher colonic absorption of deoxycholate in the elderly persons. A less efficient enterohepatic circulation of cholic acid is suggested as an underlying mechanism, whereas other factors such as enhanced colonic mucosal absorption or an increased intracolonic formation of deoxycholate are possible, but remain speculative.

Intestinal transit time in both groups were similar and therefore did not appear to be responsible for the higher deoxycholate absorption. However, a low deoxycholate absorption in young adults proved to be closely associated with a rapid gut transit,much closer than in elderly persons.

The diet of the young adults contained more vegetable proteins, carbohydrates and dietary fibre: however, differences in dietary composition

could not fully account for the enhanced deoxycholate absorption from the colon in the elderly persons.

Our data on deoxycholate absorption from the colon as a function of intestinal transit time suggest that a rapid intestinal passage may limit deoxycholate absorption only in young adulthood and not at a more advanced age. The higher colonic deoxycholate absorption in the elderly persons seems to agree with clinical data that colonic (pre) neoplastic lesions in man are rare in young adults.

CHAPTER V Colonic absorption of secondary bile-acids in patients with adenomatous polyps and in matched controls.

Exposure of colonic mucosa to bile-salt metabolites may be involved in colonic carcinogenesis. We assumed that absorption of deoxycholate from the large bowel could be a parameter of increased exposure to degraded bile-salts in patients who have an increased risk of developing colonic carcinoma.

Colonic deoxycholate absorption was investigated in eleven patients with colonic adenomas using a dual isotope dilution technique in bile and the results were compared with those obtained in eleven control subjects. Patients and controls were matched for age and intestinal transit time, since these factors can influence deoxycholate absorption. Furthermore, data were collected on the diet in both groups.

Colonic absorption of deoxycholate and the proportion of secondary bile acids in duodenal bile were significantly higher (though with some overlap of both groups) in the adenoma patients than in the control subjects. The large bowel of adenoma patients made thus a greater contribution to bile-acid conservation. It seems that this is due to increased fractional loss of cholic acid from the enterohepatic circulation. This was apparently not a consequence of the rather high cholesterol consumption in adenoma patients. These data are in agreement with the hypothesis that bacterially degraded bile-salts act as promotors of the so-called adenoma-carcinoma sequence in the human colon.

CHAPTER VI Intracolonic environment and the presence of colonic adenomas in man.

A promoting effect of large bowel contents, especially of degraded bile salts, on colonic carcinogenesis as seen in the animal model is still incompletely explored in man. Bile-salt degradation requires the presence of strictly anaerobic colon bacteria. We simultaneously investigated deoxycholate absorption (as marker of colonic exposure to tumour-promoting bile-salt metabolites), mouth-anus transit time and the ratio of anaerobic to aerobic bacteria in the stools of ten patients with colonic adenomas and of ten age-matched controls subjects.

We found that a greater predominance of strictly anaerobic bacteria was associated with more absorption of deoxycholate from the colon both in adenoma patients and in the control subjects. The anaerobic/aerobic ratio of faecal bacteria and the absorption of deoxycholate from the colon were both higher in the adenoma patients than in the control subjects. A slow gut transit appeared to enhance the growth of anaerobic more than that of aerobic bacteria in the large bowel.

We conclude therefore that colonic stasis as indicated by a slow gut transit may enhance the predominance of the anaerobic colon flora. This seems to be another factor which favours the bile-salt mediated promotion of the adenoma-carcinoma sequence in the large bowel.

CHAPTER VII Absorption of endogenous deoxycholate in relation to biliary bile-acid composition and cholesterol saturation.

It has been recognized that bile of patients with cholesterol gallstones contains a rather high proportion of deoxycholate. This has led to speculation that increased colonic absorption of deoxycholate might predispose to the formation of cholesterol stones.

The hypothesis that absorption of deoxycholate from the colon may induce cholesterol-supersaturated gallbladder bile by selective inhibition of chenodeoxycholate synthesis was tested. We therefore investigated biliary lipid and bile-acid composition in healthy subjects of various ages with considerable differences in colonic absorption of deoxycholate.

Extensive deoxycholate absorption, as noted in elderly persons, was reciprocally associated with the proportion of chenodeoxycholate in biliary bile-acids, but this was not true in young adults with less colonic absorption of deoxycholate. Deoxycholate absorption was not a major determinant of either the cholesterol saturation in bile or the proportion of cholesterol in biliary lipids.

Deoxycholate absorption and the fractional turnover rate of cholic acid were positively correlated as also indicated by the more rapid fractional turnover rate of cholic acid in elderly subjects with a rather high colonic absorption of deoxycholate. These data do not confirm the hypothesis tested. They are compatible with inhibition of chenodeoxycholate synthesis by deoxycholate absorption. However, this inhibition does not appear to be completely selective, since cholic acid metabolism also appears to be affected. The mutual interaction of colonic deoxycholate absorption and primary bile-salt metabolism needs further investigation.

8.2 Samenvatting en conclusies

HOOFDSTUK I Inleiding

Epidemiologische gegevens wijzen erop, dat invloeden vanuit onze omgeving en niet slechts genetische factoren betrokken zijn bij het ontstaan van carcinoom van de dikke darm. Met name voedingsgewoontes trekken in dit opzicht de aandacht. Aangezien het zoeken naar kankerverwekkende agentia (radioactieve straling, lucht-en waterverontreinigende producten, kunstmatige verbindingen, die aan de voeding worden toegevoegd zoals smaak-, reuk- en kleurstoffen en virale infecties) weinig aanknopingspunten heeft opgeleverd; is duidelijk geworden dat een eventueel verband tussen voeding en carcinoom van de dikke darm meer complex moet zijn.

De thans beschikbare informatie wijst veeleer op een tumorgroei bevorderend effect van de voeding. De hypothese is, dat hierbij lichaamseigen co-carcinogene stoffen zijn betrokken, die in het colon ontstaan, zoals omzettingsproducten van galzuren.

Het belangrijkste doel van het onderzoek, dat in dit proefschrift wordt beschreven, was bestudering van de deoxycholaat-absorptie uit het colon als een mogelijke graadmeter voor de blootstelling van colonslijmvlies

aan co-carcinogene afbraakproducten van galzuren. Daartoe werd de stofwisseling bestudeerd van deoxycholataat, quantitatief het belangrijkste omzettingsproduct in de gal bij de mens, en van cholzuur, het galzuur waaruit deoxycholaat wordt gevormd.

In hoofdstuk IV worden verschillen in de deoxycholaat- en cholzuurstofwisseling beschreven bij gezonde jonge volwassenen en bejaarden in relatie tot hun voedingsgewoonten en darmpassagetijden.

In hoofdstuk V wordt een vergelijking getroffen tussen patienten die adenomen van de dikke darm hebben en daardoor een verhoogde kans op carcinoom lopen, en controle personen voor wat betreft hun deoxycholaatabsorptie en cholzuurstofwisseling. De eventuele samenhang met verschillen in voeding is hierin betrokken.

In hoofdstuk VI wordt het verband beschreven tussen het anaerobe milieu in het colon, deoxycholaatabsorptie en darmpassagesnelheid zowel voor patienten met adenomen van de dikke darm als voor controle personen. In hoofdstuk VII wordt de gangbare hypothese getoetst, dat deoxycholaat absorptie uit het colon een oververzadiging van de gal met cholesterol bevordert en daardoor predisponeert tot galsteenlijden.

HOOFDSTUK II Epidemiologie en etiologie van het carcinoom van de dikke darm.

Hedendaagse inzichten in het ontstaan van carcinoom van de dikke darm worden gepresenteerd tegen de achtergrond van epidemiologie en gegevens van dierexperimenteel en klinisch onderzoek uit de literatuur. Dit hoofdstuk gaat in op de aanwijzingen voor een mogelijke rol van omzettingsproducten van galzuren als tumorgroei bevorderende stoffen in de dikke darm en het verband hiervan met voedingsgewoontes. Het is onwaarschijnlijk, dat bacteriële omzettingen van de coloninhoud niet betrokken zijn bij het ontstaan van carcinoom van de dikke darm. De galzuur hypothese legt een verbinding tussen de consumptie van een vetrijke vezelarme voeding en het frequent voorkomen van carcinoom van de dikke darm in Westerse landen. Het is tot nog toe niet bekend of veranderingen van voedingsgewoonten - in de zin van een vermindering van het vetgehalte en een toename van het gehalte aan voedingsvezel - de kans op coloncarcinoom doet afnemen.

Deze vraag zou kunnen worden beantwoord door de uitkomsten van een goed prospectief ("interventie") onderzoek, waaraan patienten met adenomen, die daardoor een verhoogde kans op coloncarcinoom hebben, deelnemen.

HOOFDSTUK III Bepaling van galzuurstofwisseling en darm passagetijd.

Galzouten zijn stoffen met vele fysiologische functies die van betekenis zijn voor de uitscheiding van cholesterol uit het lichaam (via gal en faeces) en voor de vetvertering. Ze zijn gekenmerkt door een efficiënte enterohepatische kringloop. De primaire galzuren cholzuur en chenodeoxycholzuur worden in de lever gesynthetiseerd uit cholesterol; deoxycholaat, het belangrijkste bacteriële omzettingsproduct van de galzuren in de gal, bij de mens, wordt in het colon gevormd onder invloed van anaerobe bacteriën.

De stofwisseling van de genoemde galzuren kan worden bestudeerd met isotoop dilutie technieken in gal, zoals in 1957 door Lindtstedt werd beschreven. Dit biedt de mogelijkheid de absorptie van deoxycholaat uit het colon te meten als de dagelijkse opname van deoxycholaat in de circulerende galzuur voorraad (= pool).

In dit onderzoek gebruikten wij een dubbele isotoop dilutie methode in gal voor gelijktijdige meting van cholzuur en deoxycholaat stofwisseling. De toepassing van [2,4 - ^{3}H] cholzuur en [24 - ^{14}C]deoxycholaat als galzuur merkstoffen gaf reproduceerbare resultaten.

Om de mond-anus darmpassagetijd te bepalen werden methoden gebruikt, waarbij niet absorbeerbare merkstoffen in één dosis werd toegediend. Een van de belangrijkste voordelen van deze"enkelvoudige dosis" methoden in kortdurende studies bij ambulante, niet opgenomen patienten is, dat ze eenvoudig zonder voortdurende supervisie zijn uit te voeren. Zowel vaste stoffen (polyvinyl korrels) als opgeloste stoffen (bijv. $^{51}CrCl_3$) worden toegepast. Deze keuze is echter wel van invloed op de meetresultaten: vloeibare merkstoffen worden langzamer uitgescheiden dan vaste partikels, wanneer er sprake is van een korte passagetijd. Radioactieve merkstoffen als $^{51}CrCl_3$ hebben het voordeel dat in plaats van de uitscheiding van de merkstof met de faeces ook het in het lichaam nog aanwezige gedeelte van de toegediende dosis bij herhaling kan worden bepaald, indien men de beschikking heeft over een totale lichaamsteller. Men kan met dit apparaat

tevens controleren of de faeces nauwkeurig verzameld zijn.

Het gebruik van vaste merkstoffen valt te verkiezen bij bestudering van de stofwisseling van secundaire galzuren, aangezien galzuren zich sterk hechten aan vaste faeces bestanddelen.

HOOFDSTUK IV De stofwisseling en bacteriële omzetting van galzuren bij de mens: vergelijkend onderzoek bij gezonde jonge volwassenen en bejaarden.

Er is verondersteld, dat omzetting van galzuren in co-carcinogene verbindingen een rol zou kunnen spelen bij het ontstaan van het carcinoom van de dikke darm. Omdat deze ziekte voornamelijk op gevorderde leeftijd voorkomt deden we onderzoek naar de cholzuur en deoxycholzuur stofwisseling bij elf jonge volwassenen (20 tot 30 jaar) en elf oudere personen (55 tot 75 jaar) met een dubbele isotoop dilutie methode in gal.Het voedingspakket van de deelnemers werd (volgens ieders eigen eetgewoonten) gestandaardiseerd en de darmpassagetijd werd gemeten met röntgencontrastgevende pellets en $^{51}CrCl_3$.

Uit de resultaten bleek, dat absorptie van deoxycholaat uit het colon hoger was bij de oudere personen dan bij de jonge volwassenen. Hiervoor leek ten dele de geringere efficiëntie van de enterohepatische kringloop van cholzuur verantwoordelijk, waardoor er verlies van een groter gedeelte van het cholzuur naar de dikke darm optreedt. Andere factoren zoals een betere absorptie of een toegenomen vorming van deoxycholaat zijn eveneens mogelijk, maar zijn vooralsnog speculatief.

De darmpassagetijden in beide groepen waren niet verschillend en lijken daarom op het eerste gezicht niet verantwoordelijk voor de hogere absorptie van deoxycholaat bij ouderen. Wel bleek, dat een lage absorptie aan deoxycholaat bij jonge volwassenen samenhing met een snelle darmpassage, veel meer dan bij oudere personen het geval was. De voeding van de jonge volwassenen bevatte meer koolhydraten en voedingsvezel; echter op basis van deze verschillen in voedingsgewoonten was de toegenomen absorptie van deoxycholaat uit het colon bij ouderen niet verklaarbaar.

Deze gegevens laten zien, dat een snelle darmpassage op jeugdige leeftijd, doch niet bij oudere personen(of althans veel minder) de absorptie van deoxycholaat uit het colon kan beperken.

Een hogere absorptie van deoxycholaat vanuit het colon bij oudere personen sluit aan bij het gegeven, dat carcinomen van de dikke darm en voorlopers hiervan zelden bij jonge volwassenen worden aangetroffen.

HOOFDSTUK V Absorptie van secundaire galzuren in de dikke darm bij patiënten met adenomateuze poliepen en vergelijkbare controle personen.

Het wordt van mogelijk belang geacht voor het ontstaan van carcinomen van de dikke darm, dat het slijmvlies van dit orgaan is blootgesteld aan co-carcinogene omzettingsproducten van galzuren. Wij veronderstelden, dat deoxycholaat absorptie uit de dikke darm hiervan een graadmeter zou kunnen zijn.

De absorptie van deoxycholaat uit het colon werd onderzocht bij elf patienten met adenomen van de dikke darm, die een toegenomen kans op het ontstaan van een carcinoom hebben. Hierbij maakten wij gebruik van een dubbele isotoop dilutiemethode in gal voor bepaling van de stofwisseling van cholzuur en deoxycholzuur. De resultaten werden vergeleken met die van elf controlepersonen. Beide groepen kwamen goed overeen wat betreft leeftijd en darmpassagetijd van de deelnemers, factoren die de deoxycholaat absorptie kunnen beinvloeden. Ook werden gegevens verzameld met betrekking tot de voeding in beide groepen.

Absorptie van deoxycholaat uit het colon en het gedeelte van de galzuren in de gal, dat uit omzettingsproducten bestaat waren significant hoger - zij het dat de scheiding tussen beide groepen niet absoluut was - bij de adenoom patienten dan bij de controle personen. De dikke darm droeg dus bij de adenoompatienten in sterkere mate bij tot de aanwezigheid van omzettingsproducten van galzuren in de enterohepatische kringloop. Dit bleek verband te houden met een verlies van een groter deel van hun cholzuurpool naar het colon per dag dan bij de controle personen het geval was. Deze resultaten hielden geen verband met de betrekkelijk hoge cholesterol consumptie van de adenoompatienten in vergelijking met de controle personen.

De uitkomsten zijn in overeenstemming met de hypothese, dat bacteriële afbraakproducten van galzuren de overgang van adenoom in carcinoom in het colon bij de mens bevorderen.

HOOFDSTUK VI Het milieu in de dikke darm bij patienten met adenomen van het colon.

Bij bepaalde diersoorten kunnen coloncarcinomen worden geïnduceerd door toediening van kankerverwekkende verbindingen. Hierbij is aangetoond, dat lichaamseigen stoffen uit het darmlumen, speciaal bacteriële afbraak producten van galzuren, het ontstaan van coloncarcinoom en colonadenomen kunnen bevorderen. In hoeverre bacteriële omzettingsproducten van galzuren ook bij de mens van belang zijn voor het ontstaan van carcinoom is onvolledig onderzocht. Zeker is, dat voor het ontstaan van deze producten strict anaerobe bacteriën nodig zijn.

Wij onderzochten tegelijktijd deoxycholaat absorptie uit het colon (als graadmeter voor blootstelling van het colonslijmvlies aan omzettingsproducten van galzuren), darmpassagetijd en de verhouding van anaerobe tot aerobe bacteriën in de faeces van tien patienten met adenomen in hun colon (die daardoor een verhoogde kans op coloncarcinoom hebben) en van tien vergelijkbare controle personen.

Het bleek, dat een meer anaerobe bacterieflora van de faeces samenging met een hogere absorptie van deoxycholaat uit het colon, zowel bij adenoom patienten als bij controlepersonen. De verhouding anaerobe /aerobe faeces bacteriën en de absorptie van deoxycholaat uit het colon waren beiden hoger bij adenoom patienten. Een langzame darmpassage bleek de groei van anaerobe bacteriën in de dikke darm meer te begunstigen dan die van aerobe bacteriën.

De conclusie is, dat deze gegevens steun verlenen aan de opvatting, dat ook bij de mens door een trage darm passage de rol van omzettingsproducten van galzuren bij de overgang van adenomen in carcinomen van de dikke darm kan worden bevorderd.

HOOFDSTUK VII Endogene deoxycholaatabsorptie uit het colon en de verzadiging van de gal met cholesterol.

Gal van galsteen patienten is betrekkelijk rijk aan deoxycholaat. Op grond van deze waarneming is het vermoeden geuit, dat toename van deoxycholaat absorptie uit de dikke darm het ontstaan van oververzadiging van de gal met cholesterol bevordert en daardoor de vorming van cholesterolstenen.

De hypothese dat absorptie van deoxycholaat uit het colon oververzadiging van de galblaas gal zou kunnen induceren door selectieve onderdrukking van de synthese van chenodeoxycholzuur in de lever hebben wij getoetst. Daartoe werd bij gezonde personen met een uiteenlopende absorptie van deoxycholaat en van verschillende leeftijd de gallipiden- en galzuursamenstelling van de gal en de cholzuurstofwisseling onderzocht.

Een hoge absorptie van deoxycholaat zoals werd waargenomen bij oudere personen ging samen met een laag percentage chenodeoxycholzuur in de galzuursamenstelling van de gal. Dit gold niet voor jonge volwassenen, die in het algemeen minder deoxycholaat absorbeerden uit hun dikke darm. Deoxycholaat absorptie was geen bepalende factor met betrekking tot de cholesterolverzadiging in de gal of ten aanzien van het cholesterolgehalte van de gallipiden.

Op grond van deze gegevens kunnen wij de bovengenoemde hypothese niet bevestigen. Hoewel het percentage chenodeoxycholaat omgekeerd evenredig was met de deoxycholaatabsorptie, is alleen op grond hiervan de conclusie, dat de chenodeoxycholzuur synthese door een hoge deoxycholaat absorptie wordt onderdrukt, niet mogelijk. Wel is zeker, dat een eventuele invloed van endogene deoxycholaat absorptie uit het colon op de galzuursynthese in de lever niet beperkt is tot chenodeoxycholzuur, maar dat deoxycholaat absorptie en de stofwisseling van cholzuur elkaar eveneens beinvloeden.

DANKWOORD

Bij de tot standkoming van dit onderzoek heeft de inzet van proefpersonen en patienten een grote rol gespeeld. Van vele afdelingen werd bereidwillige medewerking en hulp ontvangen, waarvoor ik zeer erkentelijk ben. Vooral zij vermeld de wezenlijke bijdrage van de diëtistes van de Kliniek voor Inwendige Ziekten; in het bijzonder Riky Lamers, die veel tijd en zorg heeft besteed aan de begeleiding van deelnemers aan het onderzoek en aan de berekening van de samenstelling van hun voeding. De staf van het Isotopen laboratorium (Hoofd: dr. F.H.M. Corstens) heeft veel werk verricht, soms ook in het weekend bij de metingen van de darmpassagetijden en is behulpzaam geweest bij de dosimetrie berekeningen. De staf van de afdeling Röntgendiagnostiek (Destijds hoofd : Prof.dr. W. Penn) is steeds bereid gevonden röntgenfoto's van faecesmonsters te maken. De medewerkers van de Afdeling Klinische Farmacie hebben mij met raad en daad bijgestaan bij het gereedmaken van de galzuur-radio-isotopen voor intraveneuze injectie. Op het Centraal Dierenlaboratorium (Hoofd: Dr. W.J.I. van der Gulden) werd de gelegenheid geboden om de (an)aerobe faeces kweken te verrichten en mochten we profiteren van de daar aanwezige ervaring in deze. Voor de hulp bij de galzuuranalyses in gal en voor de uitvoering van de (an)aerobe faeces kweken dank ik Magda Hectors en Annie van Schaik ten zeerste. De noodzakelijke literatuur informatie werd voortdurend verzorgd door de medewerkers van de Medische Bibliotheek (Hoofd: Hr.E.de Graaff). De verpleegkundige staf met name die van de Endoscopie afdeling (Hoofd: Dr. S.H. Yap) was mij steeds terwille, zo ook de medewerkers van het laboratorium Klinische Chemie (Hoofd: Prof.dr. A.P. Jansen). Metingen met de vloeistofscintillatieteller konden vlot op het laboratorium Oogheelkunde gebeuren. De illustraties zijn door de Medische Tekenkamer, in het bijzonder Hr. C. Nicolasen, en vervolgens door de Afdeling Medische Fotografie verzorgd. Tenslotte werd het typewerk met ijver door Margreet van Loon-Kamphuis en Ineke Volman verricht in Arnhem en werd het Engels gecorrigeerd door Mevr. A. Chadwick en collega R. Ponnadurai. Speciale woorden van dank komen toe op de laatste, maar niet op de minste plaats aan Ir. A.W.M. Huijbregts, Drs. J.H.M.Tuinte, F.M. Nagengast, internist, Dr. J. Bakker en Dr. J.P. Koopman.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 30 december 1945 te Meppel geboren. Hij bezocht het Triniteitslyceum te Haarlem en behaalde in 1963 het einddiploma gymnasium β. Van 1963 tot 1970 studeerde hij medicijnen aan de Katholieke Universiteit te Nijmegen en behaalde op 21 januari 1971 het artsexamen. Na een éénjarig assistentschap op de afdelingen Chirurgie (hoofden: destijds J. Drijvers en K.N. Tjia) en Gynaecologie/Verloskunde (hoofd: H. Vos) van het St.Carolus Ziekenhuis te 's Hertogenbosch en na de Nationale Tropencursus voor Artsen te Amsterdam was hij van 1972 tot 1975 werkzaam als medical officer en medical officer of health bij het Ministry of Health in Kenya met als standplaatsen Machakos en Meru.
In 1975 begon zijn opleiding tot internist aan de Universiteitskliniek voor Inwendige Ziekten te Nijmegen (destijds opleider Prof. C.L.H. Majoor). Tijdens de opleidingsperiode werd binnen de afdeling Maag, Darm en Leverziekten het onderzoek verricht, dat resulteerde in dit proefschrift (september 1978 - januari 1981).
In juli 1981 werd hij ingeschreven als internist in het specialistenregister.
Sinds augustus 1981 is hij werkzaam als wetenschappelijk ambtenaar in dienst van de Stichting Zuiver Wetenschappelijk Onderzoek op de afdeling Gastroenterologie van het Gemeente Ziekenhuis te Arnhem (hoofd: Dr. G.P. van Berge Henegouwen).

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STELLINGEN BEHOREND BIJ HET PROEFSCHRIFT VAN

S.D.J. VAN DER WERF

I De overgang van goedaardig adenoom in coloncarcinoom wordt kennelijk bevorderd door een uitgesproken anaeroob milieu in de dikke darm.

II Het effect van maatregelen tot preventie van carcinoom van de dikke darm kan worden getoetst aan het poliepvrije interval na endoscopische verwijdering van adenomateuze poliepen.

III De veronderstelling, dat diermodellen inzicht kunnen geven in de pathogenese van carcinoom van de dikke darm bij de mens staat niet vast; indien men zich realiseert, dat bij de mens kankerverwekkende stoffen niet per anum of na subcutane injectie het colon bereiken, zoals dit in het diermodel het geval is, is het zelfs twijfelachtig.

IV Voedingsadviezen bedoeld om het vóórkomen van carcinoom van de dikke darm in de bevolking te doen afnemen dienen minimaal gebaseerd te zijn op resultaten van interventie-onderzoek en niet alleen op gegevens uit de epidemiologie en/of het dierexperiment.

V Het innemen van zure of basische geneesmiddelen voor het slapen zonder hierbij voldoende te drinken kan niet alleen bij bejaarde patienten, doch ook bij gezonde jonge volwassenen hevige retrosternale pijn bij het slikken veroorzaken.

VI Verminderde ontlediging van de galblaas kan bijdragen tot vorming van lithogene galblaasgal en wel onafhankelijk van de lipiden samenstelling van de galblaasgal.

VII In de bijsluitertekst van in Nederland verkrijgbare orale anticonceptiva is ten onrechte onvermeld gebleven, dat dit medicament bij hiervoor gevoelige gebruiksters de kans op galsteenlijden kan verhogen.

VIII Totale colectomie verdient als preventief oncologische behandeling van patienten met het typische polyposis coli syndroom de voorkeur boven een subtotale colectomie.

IX Periodiek onderzoek van familieleden van patienten met polyposis coli of andere vormen van familiair voorkomend coloncarcinoom is een wijze van carcinoompreventie, die binnen onze huidige mogelijkheden ligt.

X Het gebruik van een vezelrijke voeding tijdens het verzamelen van faeces voor onderzoek op occult bloed vermindert de kans om met deze methode adenomen of carcinomen van de dikke darm op te sporen.

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XI Het merken van de identiteits polsband van HBs-antigeen positieve patienten met een rode stip is stigmatiserend.

XII Serumgalzuur spiegels zijn als leverfunctieproef te specifiek, te gevoelig en te duur.

XIII Verbetering van de kwaliteit van geneeskundig handelen kost niet in de eerste plaats meer geld, maar meer moeite.

XIV Bij bloeddonoren dient naast de HBsAg bepaling ook de anti HBc bepaling te worden verricht.

XV Een stelling bij een proefschrift hoort zo helder te zijn als gal, maar anders van kleur en niet zo bitter.